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**CLINICAL, EXPERIMENTAL AND
BIOCHEMICAL STUDIES ON THE SIGNIFICANCE OF
MONOAMINES IN PARKINSON'S DISEASE**

By

VESA SONNINEN

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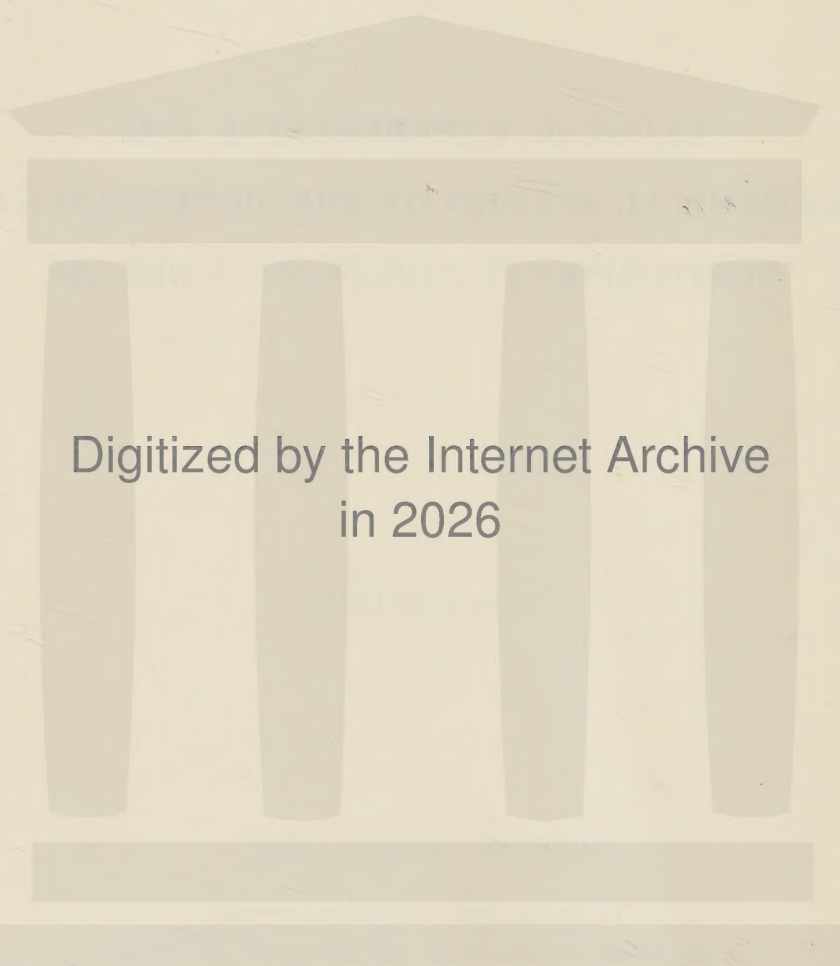
By

VESA SONNINEN

ACADEMIC DISSERTATION

To be presented with the assent
of the Medical Faculty of the University of Turku
in the large auditorium of the Medical Institute
on June 18th, 1971, at 12 o'clock noon

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I Introduction

Although Parkinson's disease, with its typical symptoms of tremor, rigidity and hypokinesia, has now been known for about two centuries, its biochemical and pathophysiological background has remained obscure until the last decade. As research methods have developed and basic knowledge about the brain grown with them, it has become clear that the essential part of the extrapyramidal system, the basal ganglia, also plays a central part in the pathophysiology of Parkinson's disease. Neuropathological investigations, for their part, very early made it clear that even if degenerative changes occur rather extensively in the brain, the most conspicuous degeneration and loss of the neurons were found in the substantia nigra (Hastler and Richert 1954, Richardson 1965).

The biochemical investigation of the extrapyramidal system became an object of intensive examination in the late 50's and early 60's. It was possible to show that, in contrast to noradrenaline, dopamine occurs in very high concentrations in the basal ganglia of the extrapyramidal system (Bertler and Rosengren 1959). Andén et al. (1964a), by employing histochemical and fluorescence technique

with an experimental pathway lesion, were able to show the pathway connections of the substantia nigra to the neostriatum. In addition they found that this pathway is dopaminergic. Neurophysiological investigation pointed out the inhibitory effect of dopaminergic neurons of the substantia nigra on the neostriatum and its central role in the function of the extrapyramidal system (McLennan 1965, Bloom et al. 1965, York 1967).

Besides dopaminergic pathways, there are serotonergic neurons in the middle parts of the brain stem connecting to the striatum. These neurons obviously play a role in the regulatory function of the extrapyramidal system (Andén et al. 1965a,b, Sourkes and Poirier 1965, Poirier et al 1967a,b). It must also be kept in mind that between the nuclei of the extrapyramidal system there is a rich cholinergic innervation which is also essential to the normal function of the system (Duvoisin 1967).

When the essential role of dopaminergic neurons in the extrapyramidal system was noted, suggestions were made as to the significance of dopamine in the pathogenesis of Parkinson's disease. Biochemical analyses showed that there was a great deficiency of dopamine in parkinsonian brains (Ehringer and Hornykiewicz 1960, Hornykiewicz 1966). Nevertheless the background is still unclear. It is not known whether the deficiency is a general change in dopamine metabolism or whether it is just a consequence of disorder or disappearance of dopaminergic neurons caused by other factors. In order to throw further light upon these questions, the present study investigates monoamine metabolism in patients with Parkinson's disease. The effect of exogenic L-DOPA injection on this metabolism and the clinical status of the patients will also be studied. A part of the results has been presented in earlier publications (Rinne and Sonninen 1968a,b,c) or congress abstracts (Rinne and Sonninen 1969 a,b, 1970 a,b).

II Review of literature

DISTRIBUTION OF MONOAMINES IN THE EXTRAPYRAMIDAL SYSTEM

Dopamine

The occurrence of dopamine, noradrenaline and 5-hydroxytryptamine in the brain has been shown by biochemical and histochemical methods. These substances have been noted in the brain of all mammals examined, including man (Carlsson et al. 1958, Carlsson 1959, Sano et al. 1959, Bertler and Rosengren 1959 a,b, Ehringer and Hornykiewicz 1960, Bertler 1961a, Lavery and Sharman 1965a).

Approximately 50 % of the total catecholamine metabolism in the human brain is dopamine metabolism (Carlsson et al. 1957 a, Sano et al. 1959). This has supported the view that dopamine is an independent transmitter and not only the precursor of noradrenaline. When the local distributions of noradrenaline

and dopamine are compared, it is noted that neurons containing dopamine and noradrenaline are localized in different parts of the brain. Dopamine occurs most abundantly in the nuclei of the extrapyramidal system, nucleus caudatus and putamen, and less in the substantia nigra and globus pallidus. Noradrenaline concentration is relatively low in these areas. The nucleus caudatus contains $0.1 \mu\text{g/g}$ tissue noradrenaline, whereas the hypothalamus contains $1-2 \mu\text{g/g}$. The corresponding dopamine concentrations are $2 - 10 \mu\text{g/g}$ and $0.1 - 0.2 \mu\text{g/g}$. It is a fact that the highest noradrenaline concentration is met in the hypothalamus. Other areas with a high noradrenaline concentration are found in the brain stem (Bertler and Rosengren 1959, Carlsson 1959, Ehringer and Hornykiewicz 1960, Bertler et al. 1964, Hornykiewicz 1966).

A great number of dopamine-containing neurons in the brain form the important nigro-neostriatal pathway. The cell bodies of these neurons are primarily localized in the zona compacta of the substantia nigra, but they are also present in the adjacent mesencephalic reticular formation. By combining histochemical and biochemical studies of experimental lesions it has been possible to demonstrate in laboratory animals that these substantia nigra neurons ascend in the ventral crus cerebri and lateral portion of the forebrain bundle, terminating in the neostriatum. There, these nerve fibers form a dense meshwork of very fine terminal fibers (Andén et al. 1964a, 1965a,b, Fuxe et al 1966). Evidence of the existence of the dopaminergic pathways of substantia nigra in the internal capsule has been obtained in human autopsy material by analysing the concentration of homovanillic acid (HVA), the main metabolite of dopamine (Hornykiewicz et al. 1968).

Of the other mesencephalic dopamine-containing neurons, the cell bodies localizing around interpeduncular nucleus innervate

the nucleus accumbens, septal nuclei and tuberculum olfactorium (Andén et al. 1966b). In the hypothalamus, on the other hand, dopaminergic neurons form a prominent tubero-infundibular system. Cell bodies of these neurons mainly localize in the arcuate and anterior periventricular nucleus, sending axons into the median eminence (Fuxe 1964, Fuxe and Hökfelt 1966, Rinne et al. 1967, 1968d). Furthermore, dopaminergic neurons have been demonstrated within the retina (Malmfors 1963).

Noradrenaline

It has been demonstrated that noradrenaline-containing cell bodies are localized mainly in the pons and medulla oblongata (Dahlström and Fuxe 1964, 1965). Clearcut descending bulbospinal noradrenergic pathways have been shown to begin from these cell bodies (Carlsson et al. 1964, Dahlström and Fuxe 1965).

Cell bodies sending large ascending dorsal noradrenergic pathways are mainly localized in locus coeruleus. The axons of these nerve cells project rostrally, innervating the thalamus, cortex cerebri, hippocampal formation and cerebellum (Fuxe 1965a,b, Andén et al 1966 b). Noradrenaline-containing cell bodies in the medulla oblongata and pons also ascend as the ventral noradrenergic pathways ending mostly in the hypothalamus and in the preoptic and septal area of the limbic system. Some axons of these cell bodies also enter the cortex cerebri and cerebellum (Andén et al. 1964 a , Fuxe et al. 1968).

5-Hydroxytryptamine

The 5-HT-containing neurons also have a typical localization

in the brain. Cell bodies of these neurons are mainly localized in the raphe nuclei of the lower brainstem (Dahlström and Fuxe 1964). The neurons of mesencephalic raphe cell groups give rise to the ascending 5-HT pathways which project via the medial fore-brain bundle to the lateral hypothalamus, septal nuclei, amygdaloid nucleus and hippocampus, as well as to the cerebral cortex (Andén et al. 1966b). Instead of a moderate number of 5-HT nerve terminals in globus pallidus, the neostriatum of the rat seems to receive no clearcut 5-HT innervation (Fuxe 1965a,b). Experimental data, however, indicate that the caudate nucleus and putamen in the cat also receive serotonergic nerve fibres from the brainstem (Poirier et al. 1966, 1967a,b).

From the more caudal groups of raphe nuclei located in the medulla oblongata, 5-HT-containing bulbospinal pathways descend to the lumbal and sacral part of spinal cord (Carlsson et al. 1964, Dahlström and Fuxe 1965).

EXPERIMENTAL STUDIES ON MONOAMINES IN THE EXTRAPYRAMIDAL SYSTEM

D r u g s i n t e r f e r i n g w i t h m o n o a m i n e s

Reserpine, butyrophenones and phenothiazines

Evidence of the significance of dopaminergic neurons in the function and pathophysiology of the extrapyramidal system has been obtained from experiments in which disorders of this system were induced by drugs interfering with the monoamine metabolism. Of these drugs, reserpine is that which most clearly affects the monoamine metabolism of the brain. At the same time it has an extremely disturbing influence upon the extrapyramidal system, producing in animals rigidity and akinesia, with catalepsy as the extreme form (Bein 1956, Carlsson et al. 1957a,b, Carlsson 1965). In man it has been noted to produce a syndrome similar to Parkinson's disease, in which tremor, rigidity and akinesia dominate ; these symptoms occur very often with high doses of reserpine (Delay 1956).

The pharmacological effect of reserpine is based on the depletion of monoamines (Carlsson et al. 1957b, Bertler 1961b, Tracka and Carlsson 1967). Because only dopamine plays a central role in the extrapyramidal system, the effect is based on the depletion of dopamine (Carlsson et al 1957b, Bertler 1961b, Carlsson 1965). In addition, L-DOPA eliminates the hypokinesia and catalepsy caused by reserpine, restoring motor activity (Carlsson et al. 1957a,b, Blaschko and Chruskiel 1960, Degkwits et al. 1960), whereas 5-HT does not have this effect (Carlsson et al. 1957b). On the cellular level, reserpine inhibits dopamine uptake in the storing granules (Smith 1962, Hamberg et al. 1964, Malmfors 1965, Lundborg 1967,

Carlsson and Lindqvist 1967).

Like reserpine, many phenothiazines cause disorders in the extrapyramidal system. Their biochemical effect is not wholly known. However, large doses of e.g. chlorpromazine have been demonstrated to increase the concentration of HVA in the brain (Laverty and Sharman 1965b, O'Keefe et al. 1970) and cerebrospinal fluid (Guldborg and Yates 1968, 1969, Roos 1969). On the other hand, it either has no influence on the dopamine concentration of the brain (Holzer and Hornykiewicz 1959, Gey and Pletscher 1964a) or else causes a slight decrease (Laverty and Sharman 1965b). There is also evidence of increased turnover of brain dopamine caused by chlorpromazine without changes in that of noradrenaline (Neff and Costa 1967).

Dopamine receptors are also blocked by drugs such as haloperidol (Van Rosum 1966, Yeh et al. 1969), bulbocapine (Ernst 1969), and many phenothiazines (Hornykiewicz 1966). It is possible that these drugs increase turnover of dopamine through a feedback mechanism due to the blocking of the dopamine receptors in the brain (Carlsson and Lindqvist 1963, da Prada and Pletscher 1966). On the other hand, stimulation of dopamine receptors with apomorphine decreases dopamine turnover in the brain (Roos 1969).

L-DOPA

In experimental animals, it has been possible to show that L-DOPA increases dopamine concentration in the brain without significantly influencing the amount of noradrenaline (Bartholini and Pletscher 1968, Butcher and Engel 1969, Everett and Bocherding 1970, Wurtman et al. 1970, Hyypä et al. 1971). In addition, L-DOPA elevates HVA, dihydroxyphenylacetic acid and 3-O-methyldopa

concentrations in the brain (Pletscher et al 1970, Kuruma et al. 1970). After L-DOPA injection, catecholamines and phenolcarbon acids reach their maximum concentrations quickly and disappear relatively soon, but 3-O-methyldopa remains considerably longer than the others (Pletscher et al. 1970, Kuruma et al. 1970). On the other hand, the 5-HT concentration somewhat decreases in the brain (Bartholini et al. 1968, Butcher et al. 1970, Everett and Borcherding 1970).

In man, direct evidence of the influence of L-DOPA on the brain monoamines is scanty compared with the experiences in animals. However, it has been demonstrated recently that in a parkinsonian patient L-DOPA elevates the dopamine and HVA concentrations in the brain without significant effect on the concentration of noradrenaline (Rinne et al. 1971). An increased dopamine concentration in the human brain was suggested also by the increased HVA (Weinert et al. 1969, Curzon et al. 1970, Rinne et al. 1970 a,b, Van Woert and Bowers 1970) and 3-O-methyldopa (Sharpless et al. 1971) concentrations in the cerebrospinal fluid (CSF) during L-DOPA treatment. On the other hand, during L-DOPA treatment urinary excretion of dopamine (Cotzias 1969, Goodwin et al. 1970), HVA (Calne et al. 1969a,b, Tyce and Muentzer 1970) and dihydroxyphenylacetic acid (Calne et al. 1969a, O'Gorman et al. 1970) is greatly increased. Furthermore, excretion of vanilmandelic acid was slightly increased (Tyce et al. 1970).

There is much experimental evidence that in animals L-DOPA causes syndromes involving both the peripheral and central nervous system (Hornykiewicz 1966, Pletscher et al. 1970). In human parkinsonian patients parenteral administration or small oral doses of L-DOPA have a beneficial but temporary effect on akinesia (Birkmayer and Hornykiewicz 1961, Barbeau et al. 1962, Gerstenbrand and Patensky 1962, Friedhoff et al. 1963, Gerstenbrand et al. 1963).

Some other investigators failed to find any significant effect (Greer and Williams 1963, McGeer and Zeldowicz 1964, Fehling 1966, Aebert 1967). However, during recent years an increasing number of clinical studies have been published by several neurological centers indicating that large oral doses of L-DOPA relieve the symptoms of patients with Parkinson's disease (Cotzias 1968, et al. 1969, Yahr et al. 1969, Barbeau 1969, Birkmayer 1969, Calne et al. 1969, Goodwin-Austen 1969, Klawans and Garvin 1969, Siegfried et al. 1969, Tissot et al. 1969, Kaesar 1969, Kaufmann et al 1970, Mawdsley 1970, McDowell et al. 1970, Rinne et al. 1970a,b).

Experiments interfering with monoamine neurons of the extrapyramidal system

The central role of the nigro-striatal dopaminergic pathway is further substantiated by the fact that lesion of the substantia nigra or nigro-striatal nerve fibres causes a marked loss of dopamine in the ipsilateral striatum (Bertler et al. 1964, Poirier and Sourkes 1965, Poirier et al. 1966, 1967b). Furthermore, lesion of nigro-striatal nerve fibres also causes decrease in the activity of thyrosine hydroxylase (Goldstein et al. 1966, Poirier et al. 1969) and dopa decarboxylase (Goldstein et al. 1969, Lancaster et al. 1970) in the ipsilateral striatum. On the other hand, stimu-

lation of the substantia nigra has resulted in the production of dopamine in the neostriatum (McLennan 1965).

In the monkey, a lesion in the upper brain stem causing a severe loss of nerve cells in the pars compacta of the ipsilateral substantia nigra is followed by a decrease in dopamine and nor-adrenaline in the ipsilateral striatum. This may result in a disturbance of motor activity of the contralateral limbs (Sourkes and Poirier 1966, Poirier et al. 1967b). In the rat, on the other hand, unilateral lesion of the substantia nigra-neostriatum dopamine neurons in itself produced no pronounced asymmetries. In contrast, injection of neuroleptic drugs such as reserpine, chlorpromazine and haloperidol did cause manifest unilateral extrapyramidal symptoms in rats with a lesion (Andén et al. 1966b, Andén 1967a,b).

Electrophysiological findings after microelectrophoretic injection of dopamine have shown that dopamine has predominantly an inhibitory effect on neurons of the caudate nucleus (Bloom et al. 1965, McLennan and York 1967, York 1967).

Interruption of motor pathways in the dorso-medial cerebral peduncle leads to decreased concentration of 5-HT (Poirier et al. 1967a,b, Poirier et al. 1969) and diminished activity of tryptophan hydroxylase (Poirier et al. 1969) in the ipsilateral striatum. On the other hand, implantation of 5-HT in the rostroventral part of the corpus striatum induced gnawing movements while implantation of 5-HT or 5-hydroxytryptophan into the substantia nigra caused tremor (Hadzovic and Ernst 1969).

MONOAMINE METABOLISM IN PARKINSON'S DISEASE

B r a i n m e t a b o l i s m o f m o n o a m i n e s

Evidence of the significance of dopamine for the biochemical pathogenesis of Parkinson's disease was obtained when it was found that a great deficiency of dopamine in the striatum and substantia nigra was typical of this disease (Ehringer and Hornykiewicz 1960, Bernheimer and Hornykiewicz 1964, 1965). Decrease of dopamine was noted in parkinsonian patients of different etiologies, although it was more severe in the postencephalitic variety. Furthermore, the degree of striatal dopamine deficiency correlated well to the degree of cell loss in the pars compacta of the substantia nigra (Ehringer and Hornykiewicz 1960, Hornykiewicz 1963). In addition, in a patient with hemiparkinsonismus decrease of dopamine concentration was marked in the striatum contralateral to the motor symptoms (Barolin et al. 1964).

Deficiency of dopamine is associated with decrease of HVA in the nigra-striatal system, although not to the same extent as decrease of dopamine Bernheimer and Hornykiewicz 1965, Gottfries et al. 1965). The ratio of dopamine to HVA is about one in the normal human striatum, but in the parkinsonian striatum it was shifted in favour of HVA. It has been suggested that, as a compensatory phenomenon, neurons still remaining in the striatum have been turned over faster than in the normal brain (Hornykiewicz 1966).

It has been noted that the concentration of hypothalamic nor-adrenaline (Ehringer and Hornykiewicz 1960, Bernheimer et al. 1963b)

and of 5-HT (Bernheimer et al. 1961) in the parkinsonian brain are also decreased, but this is not so severe as with dopamine. Moreover, these concentrations are returned to the normal level following a monoamine oxidase inhibitor, whereas dopamine deficiency in the striatum remains unaffected (Bernheimer et al. 1963,a,b).

Monoamine enzymes

The activity of tyrosine hydroxylase, the enzyme participating in the dopamine synthesis, is decreased in the striatum of animals with an experimental mesencephalic lesion interrupting the nigro-striatal pathways (Goldstein et al. 1966). Nevertheless, no investigations of this enzyme in the parkinsonian brain have been carried out.

Earlier investigations of the activity of dopa decarboxylase in the parkinsonian brain did not show any significant change when compared with the control series (Bernheimer and Hornykiewicz 1963). However, recent studies with more sensitive methods have demonstrated decreased activity in the parkinsonian brain (Metzel et al. 1970). The decrease in the activity of this enzyme was greatest in the striatum, less pronounced in the hypothalamus but unchanged in the cerebellar and cerebral cortex (Lloyd and Hornykiewicz 1970).

Studies on the activities of dopamine- β -hydroxylase and monoamine oxidase have shown roughly normal values (Bernheimer and Hornykiewicz 1962, Metzel et al. 1970).

There are no studies on possible changes of catechol-o-methyltransferase in the parkinsonian brain.

Acetylcholinesterase

Little information is available on changes in acetylcholinesterase activity in Parkinson's disease. Weber (1952) suggests a lack of cholinesterase in the putamen, pallidum and nucleus ruber in two autopsy cases of Parkinson's disease, but no differentiation was made between specific types of cholinesterases measured.

Monoamine metabolites in the cerebrospinal fluid

Of the monoamines and their metabolites in the human cerebrospinal fluid (CSF) HVA (Andén et al. 1963), 5-hydroxy-indoleacetic acid (5-HIAA) (Ashcroft and Sharman 1960, Ashcroft et al. 1966) and recently 3-methoxy-4-hydroxyphenylglycol (MOPEG) (Schanberg et al. 1968) and noradrenaline (Häggendahl 1966) in small amounts have been demonstrated.

There is experimental evidence indicating that changes in the CSF level of monoamine metabolites reflect changes of the parent amines in the brain, if elimination of these acid monoamine metabolites from the brain and CSF is intact (Bartholini et al. 1966, Fletscher et al. 1967, Guldberg and Yates 1968, 1969, Andersson and Roos 1968, Eccleston et al. 1968, Moir et al. 1970). There is evidence of some age-dependent variation in the concentration of acid monoamine metabolites in the CSF. Bowers and Gerbode (1968)

have shown that in psychiatric and neurologic patients there was a U-shaped relationship between age and content of 5-HIAA in the CSF, the youngest and oldest having higher levels than the middle-aged patients. Correspondingly, higher HVA levels were also found in the old-age group. Gottfries et al. (1970) did not find a similar relationship in healthy volunteers or psychiatric patients nor did they find a significant positive correlation between age and both 5-HIAA and HVA. Papeschi et al. (1970) did not find any significant difference between ventricular HVA and the age of parkinsonian patients.

A decrease of HVA in lumbar CSF has been found in parkinsonian cases (Johansson and Roos 1967, Bernheimer et al 1966, Barolin, and Hornykiewicz 1967, Rinne and Sonninen 1968a,b), as has a decrease in ventricular CSF (Guldborg et al. 1967, Papeschi et al. 1970). There is also a less marked decrease in the concentration of 5-HIAA (Johansson and Roos 1967, Gottfries et al. 1969). Furthermore, if the elimination of these acids from the CSF is inhibited by probenecid (Guldborg et al. 1966), the increase in concentration is slower in the CSF of parkinsonian patients than in controls (Olsson and Roos 1968). However, low levels of acid monoamine metabolites in the CSF are not peculiar to Parkinson's disease. Reduced concentrations have been demonstrated in other neurologic diseases (Barolin and Hornykiewicz 1967, Papeschi et al. 1970, Brody et al. 1970) and psychiatric disease conditions (Dencker et al. 1966, Bowers et al. 1969, Gottfries et al. 1969).

U r i n a r y e x c r e t i o n o f m o n o a m i n e s

Urinary excretion of dopamine in parkinsonian patients has given contradictory results. Barbeau et al. (1961, 1962) reported decreased urinary excretion of dopamine in parkinsonian patients. In some but not all later studies, these findings have been confirmed (Bishoff and Torres 1962, Weil-Malherbe and van Burren 1969). For example, O'Reilly et al. (1965) and Smith and Kellow (1969) found increased excretion of dopamine in patients with Parkinson's disease and with other extrapyramidal disorders characterized by involuntary movements.

Furthermore, elevated excretion of dopamine has been described after thalamotomy (Westlake and Tew 1966) though Schneiden and Williams (1970) did not confirm this finding. Correspondingly, normal urinary excretion of HVA has been noted after thalamotomy (Rinne et al. 1967). Contrary to the finding of Simek (1968), some investigators have not observed any significant decrease in urinary excretion of HVA (Westlake and Tew 1966, Greer and Williams 1963, Rinne et al. 1967, Calne et al. 1969a), or of other alcoholic or acid catecholamine metabolites (Calne et al. 1969a) in parkinsonian patients compared with non-parkinsonian controls.

The excretion of adrenaline and noradrenaline has generally been noted to remain unchanged in Parkinson's disease (Barbeau et al. 1961, Nashol and Kirschner 1963). Correspondingly, a normal amount of the main metabolite of adrenaline and noradrenaline, vanilmandelic acid (VMA), has been noted in the urine of parkinsonian patients (Rinne et al. 1967).

Diminished urinary excretion of 5-HT has also been found in Parkinson's disease (Barbeau et al. 1963b). However, this has not

been confirmed in other studies (Resnik et al. 1962, O'Rayley et al. 1965). Furthermore, Resnik et al. (1962) reported that, following 5-hydroxytryptophane loading, the excretion of 5-HT and 5-HIAA were normal in parkinsonian patients. Increased excretion of 5-HIAA occurs shortly after stereotaxic thalamotomy (Westlake and Tew 1966).

Some recent findings have suggested a possible role of 3,4-dimethoxyphenylethylamine (DMPEA) in the pathogenesis of Parkinson's disease. Ernst (1962, 1965) and Barbeau (1966) were able to produce a hypokinetic, rigid syndrome in animals with this compound. Furthermore, a large amount of this substance has been found in the urine of parkinsonian patients as a "pink spot" demonstrated by the paper chromatographic technique (Barbeau et al. 1963a).

It was reported originally that DMPEA determined by this method was unique to the urine of schizophrenic patients, suggesting a close relationship between the compound and the disease (Friedhoff and van Winkle 1962, Kuehl et al. 1964, Bourdillon et al. 1965). Nevertheless DMPEA was also found in the urine of non-schizophrenic normal patients (Takezada et al 1963) and later attempts to detect the compound have been unsuccessful (Perry et al. 1964, Faurbye and Pind 1964). Furthermore, doubt has been cast on the identification of the compound as DMPEA by the methods used (Boulton and Felton 1966, Nunn and Wheeler 1966). In order to increase the sensitivity of identification of the compound as DMPEA, the paper chromatographic system was combined with a fluorimetric examination, and many of the "pink spots" of the paper chromatographic method did not fluoresce (Bell and Somerville 1966, Boulton et al. 1967). It has been shown that the "pink spot" consists of a number of components (Boulton et al 1967, Kuehl et al. 1968, Perry et al. 1967). It was not possible by

this technique to establish any significant difference in urinary excretion of DMPEA between parkinsonian patients and control subjects (Rinne and Sonninen 1967).

There is experimental evidence that DMPEA is metabolized into 3,4-dimethoxyphenylacetic acid (DMPAA) (Friedhoff et al 1966, Wagner et al. 1966). Similarly DMPAA can be formed from HVA (Faurbye and Pind 1966). DMPAA has been noted in the urine of normal subjects (Kuehl et al. 1966, Friedhoff and Furia 1967), but there is no knowledge of the urine excretion of this compound in parkinsonian patients.

III Purpose of the present study

The subject of this investigation was to clarify the significance of monoamine metabolism in Parkinson's disease and its correlation with the clinical severity of the disease. To this end, the following main points were studied:

1. The basal urinary excretion of monoamines and their metabolites.
2. The concentration of the acid monoamine metabolites in the CSF.
3. The metabolism of an exogenously administered single injection of L-DOPA into the urine and the CSF.
4. The effect of probenecid treatment on the concentration of acid monoamine metabolites in the CSF.
5. The correlation of urinary excretion of monoamines and their metabolites and the concentration of acid monoamine metabolites in the CSF, with various clinical aspects.
6. Clinical response to a single injection of L-DOPA.

IV Patients and methods

PATIENTS

P a r k i n s o n i a n p a t i e n t s

The series of patients whose urine was analysed consisted of 102 subjects. Their main features are summarised in Table 1.

The series of patients whose cerebrospinal fluid was examined consisted of 157 subjects. Seventy-six of these also belong to the first group. The main findings of these patients are presented in Table 2.

The double blind trial was carried out on 36 parkinsonian patients in order to discover the effect of a single injection of L-DOPA on parkinsonian symptomatology. The main characteristics of these patients are shown in Table 3.

There was no doubt as to the diagnosis in any of the cases.

Table 1. The main features of the parkinsonian patients.

Group	P a t i e n t s							Duration of disease (years)	Disability (NUDS)
	Total number	Sex		Age (years)	Side of operation				
		F	M		right	left	bilat.		
All cases	102	47	55	60 ± 0.9	23	19	8	8.5 ± 0.7	25 ± 2.5
Female	47	47	-	59 ± 1.2	10	8	1	8.6 ± 1.1	25 ± 3.8
Male	55	-	55	60 ± 1.2	13	11	7	8.3 ± 0.9	25 ± 2.8
Idiopathic	77	38	39	59 ± 1.0	15	15	6	6.8 ± 0.7	30 ± 2.7
Postencephalitic	25	8	17	60 ± 1.8	8	4	1	10.1 ± 1.6	19 ± 2.9
Non-operated	52	28	24	59 ± 1.3	-	-	-	5.2 ± 0.6	24 ± 3.8
Operated	50	36	14	60 ± 1.0	23	19	8	11.7 ± 1.1	26 ± 2.4
Controls	48	18	30	56 ± 1.3	-	-	-	-	-

Table 2. The main features of the parkinsonian patients.

Groups	Total number	P a t i e n t s						Duration of disease (years)	Disability (NUDS)
		Sex		Age (years)	Side of operation				
		F	M		Right	Left			
						Bilat.			
All cases	157	89	68	59 ± 0.7	31	25	10	7.9 ± 0.5	28 ± 1.7
Female	89	89	-	59 ± 0.9	19	12	4	8.0 ± 0.6	27 ± 2.0
Male	68	-	68	59 ± 1.2	12	13	6	7.7 ± 0.8	29 ± 2.9
Idiopathic	130	73	57	59 ± 0.8	25	21	10	7.8 ± 0.5	29 ± 1.9
Postencephalitic	27	16	11	60 ± 1.7	6	4	-	8.3 ± 1.4	25 ± 3.2
Non-operated	91	54	37	61 ± 1.0	-	-	-	7.0 ± 0.6	30 ± 2.7
Thalamotomized	66	35	31	57 ± 1.0	31	25	10	9.1 ± 0.8	26 ± 2.0

All had been followed up, most of them for many years, in neurological or neurosurgical departments. Some of the parkinsonian patients had been submitted to stereotaxic thalamotomy 1-14 years (on average 2 years) earlier.

Table 3. Some characteristics of the parkinsonian patients

Group	Sex		Total number	P a t i e n t s			Duration of disease (years)	Dis- ability (NUDS)	
				Age (years)	Side of operation				
	F	M			Right	Left	Bilat.		
Non-operated	8	8	16	55 $\pm$ 3	-	-	-	5.0 $\pm$ 3.3	24 $\pm$ 2
Operated	8	12	20	60 $\pm$ 3	9	7	4	12.6 $\pm$ 2.5	27 $\pm$ 1
Total	16	20	36	58 $\pm$ 2	-	-	-	9.2 $\pm$ 2.0	26 $\pm$ 1

C o n t r o l p a t i e n t s

The control group for urine analyses consisted of 48 patients, 18 of whom were women, 30 men. They suffered from various neurological disorders without extrapyramidal signs : vascular disorders in 10 cases, neuromuscular disorders in 10, as well as degenerative disorders of the spinal cord in 12, post-traumatic encephalo-

pathy in 5 and other disorders in 11 cases. The mean age of this group was 56 ± 1.3 years.

The control series for the CSF studies consisted of 63 neurological patients without extrapyramidal disorders. Twenty-six of these patients also belonged to the above-mentioned control group used in urine analyses. The mean age of these patients was 59 ± 0.1 years.

General control system of the patients

All subjects were in-patients at the time of examination. Function of liver and kidneys were examined in order to eliminate possible errors. At least two days prior to the urine or CSF collection the patients were under controlled conditions. They were kept on a diet excluding tobacco and excluding food or drugs known to interfere with monoamine metabolism or with the determination methods (Euler et al. 1956, Andersson et al. 1958, Westfall and Watts 1964, Carruthers et al. 1970). Otherwise the patients were on normal hospital diet. In the parkinsonian patients from whom a urine or CSF sample was taken, antiparkinsonian drugs were discontinued 3 days before the examination. On the days when urine or CSF was collected the patients were kept in bed.

U r i n e a n d c e r e b r o s p i n a l f l u i d c o l l e c t i o n

A 24-hour urine collection was made on three successive days. A 10-20 ml sample of CSF was obtained by lumbar puncture carried out in the lateral position. Both the urine and CSF samples were collected in hydrochlorid acid (pH 2-3) and stored deep-frozen until analysed.

CLINICAL ASSESSMENT

Prior to the urine and CSF collection, a careful clinical examination was performed. The disability of the patients was estimated by using the Northwestern University Disability Scale (NUDS) as described by Canter et al. (1961). An evaluation of the degree of parkinsonian symptomatology was assessed according to a wide range of physical findings made at a neurological examination and rated on a five point scale with criteria similar to those employed by Yahr et al. (1968, 1969).

In addition, tremor was measured quantitatively as described by Clarke et al. (1966). The transducer was fixed to the hand and movement was recorded on an electrocardiographic recorder with the paper running at the rate of 1.0 cm per second. The amplitude of all tremor oscillation was measured during ten seconds and mean amplitude was then calculated.

Furthermore, the following performance test was carried out. During a period of 30 seconds the patient was asked to make the following movements as rapidly as possible: (1) finger to nose, (2) heel to knee, (3) pronation-supination using a ball-bearing mounted disk to control the range of the movement, (4) picking up pins from a bowl and placing them in the holes of a perforated plate, (5) pressing the key of a digital counter with the forefinger. (6) The patient was asked to walk a distance of 30 meters and do an about-turn midway, the time needed for this being recorded, (7) the patient's initiation transit and total reaction time to sound and light stimuli were measured by a modification of the system described by Barbeau (1966).

EXPERIMENTS

L - D O P A l o a d i n g t e s t

L-DOPA loading was carried out in parkinsonian patients and control patients on whom urine analyses were performed. On the second day of urinary collection 1.5 mg/kg b.w. of L-DOPA (F. Hoffman, LaRoche & Co, Basle Switzerland) in a 0.5 per cent solution was given by slow intravenous injection during 10 min. The injection was always given at the same time of day 12.00 hours $\pm$ 30 min. For CSF studies the second lumbar puncture was carried out on the second urinary collection day eight hours after the L-DOPA injection.

P r o b e n e c i d t e s t

The probenecid test was carried out according to the method of Roos and Sjöström (1969) by giving 1 g probenecid orally twice a day for two days and 2 g in the morning of the third day followed by the second lumbar puncture six hours later.

A double blind trial on the
effect of a single injection
of L - D O P A

A double blind trial was employed. The patients were given L-DOPA or physiologic saline by intravenous injection on two successive days. The order of drug administration varied. The patients were told that they were receiving the same drug on both days. The drugs were injected by others and the author then performed the examination without knowing which substance the patient had received. The injection of 1.5 mg/kg b.w. of L-DOPA or a corresponding amount of physiological saline was carried out as described above. The clinical assessment of the patients was performed prior to treatment and 30 min and 6 h afterwards in the same quiet room. The findings were rated by the methods described above.

BIOCHEMICAL ANALYSIS METHODS

D e t e r m i n a t i o n o f u r i n a r y
d o p a m i n e

Dopamine in urine was measured by Uuspää's modification of the method described by Anton and Sayre (1964). For the separation of dopamine from its metabolites a Dowex Wx4 (mesh 200-400 mesh) was employed. After this the fluoroform formation was carried out by using periodate, and the fluorescence was read at activation and fluorescence wavelengths 330 nm and 384 nm. Recovery of dopamine in this method was 75.5 ± 3.0 %.

D e t e r m i n a t i o n o f u r i n a r y
a d r e n a l i n e a n d n o r a d r e n a l i n e

Adrenaline and noradrenaline were extracted from urine at pH 8.5 with activated aluminum oxide in a test tube and eluted with 0.1 N hydrochloric acid. Fluorescence development was carried out with potassium ferricyanide, and the fluorescence intensity of fluoroform of adrenaline and noradrenaline was determined by a Hitachi fluorospectrophotometer with a primary wave-length of 400 nm

and 436 nm and a secondary wave-length of 520 nm (Manninen 1969). Recovery in this method was 78.0 ± 4.0 %.

D e t e r m i n a t i o n o f 3 - m e t h o x y - 4 - h y d r o x y m a n d e l i c a c i d

This compound was measured by the method of Pisano (1962). Initial attempts to determine it by this method were based on oxidation to vanillin by sodium metaperiodate. The fluorescence was read at A 330 nm and A 347 nm and F 370 nm. Recovery by this method was 80.0 ± 2.3 %.

D e t e r m i n a t i o n o f m e t a d r e n a l i n e a n d n o r m e t a d r e n a l i n e

Fluorimetric determination of 3-O-metylated derivatives of adrenaline and noradrenaline in urine was performed first by using a aluminum oxide column and then by using an Amberlite CG 130 column.

The eluate (0.5 N hydrochloric acid) was oxidized by potassium ferricyanide and fluorescence was read at two different fluorescence frequencies : A 390 nm and F 500 nm and A 430 nm and F 520 nm. The calculation was based on the Brunjes method (1962). Recovery was $72.9 \pm 3.0 \%$.

D e t e r m i n a t i o n o f h o m o v a n i l l i c a c i d i n u r i n e

First HVA was adsorbed into Dovex AG-1 resin in column, and then the 1N hydrochloric acid eluate obtained was extracted by chloroform-toluene. The HVA was oxidized by potassium ferricyanide in alkali in order to obtain the fluorescent product, which was then measured at the wavelengths A 320 nm and F 420 nm (Sato 1965). Recovery was $79.3 \pm 4 \%$ by this method.

3 , 4 - D i m e t h o x y p h e n y l e t y l a m i n e

DMPEA was extracted from urine by chloroform and then paper-chromatographed (Friedhoff et al. 1963). The pink spot

obtained was chromatographed by two dimensional thin layer chromatography (Silica Gel G'E. Merk) and stained with ninhydrin. (Kuehl et al. 1964).

3 , 4 - D i m e t h o x y p h e n y l a c e t i c a c i d

DMPAA was extracted from urine by chloroform and purified by two successive extractions and by thin layer plate chromatography and after an esterification was determined by gas chromatography by using the column F-60-Z in 150°C. The length of the column was 6 feet (Wagner et al. 1966). Recovery was $89.3 \pm 3.1 \%$.

A m e t h o d o f m e a s u r i n g 5 - h y d r o x y - i n d o l e a c e t i c a c i d i n u r i n e

Ketone acids and indolylacetic acid were removed from urine by extracting them with organic dissolvents. Remaining urinary 5-HIAA was determined colorimetrically by using 1-nitroso-2-naphtole as a color reagens (Udenfriend et al. 1958). Recovery was $85 \pm 4.2 \%$.

D e t e r m i n a t i o n o f h o m o v a n i l l i c a c i d i n t h e c e r e b r o s p i n a l f l u i d

HVA was analysed by the method of Andén et al. (1963). HVA was extracted from deproteinized cerebrospinal fluid by ethyl-acetate instead of ether and oxidized by potassium ferricyanide in alkali. The fluoroform was read at the wavelengths A 320nm and F 420nm. Recovery in this method was $88.0 \pm 4.0 \%$.

5 - H y d r o x y i n d o l e a c e t i c a c i d i n t h e c e r e b r o s p i n a l f l u i d

5-HIAA was measured by the method of Ashcroft and Sharman (1960) with duplicate samples and an internal standard for each assay. The proteins were precipitated by zinc sulfate and sodium-hydroxide, and the indole compound developed in hydrochloric acid was determined by fluorospectrophotometry at the wavelengths A 365 nm and F 410 nm. Recovery was $89.1 \pm 4.0 \%$.

T h e d e t e r m i n a t i o n o f u r i n a r y c r e a t i n i n e

The urinary creatinine was determined by using the reagent of Lloyd and sodium picrate. The intensity of the color was measured spectrophotometrically (Camara et al. 1951).

STATISTICAL TREATMENT

The mean and standard error were calculated for statistical analyses of the results. The student's t-test and Chi-Square tests were used.

The difference between two means was said to be significant if $0.01 < P < 0.05$, highly significant if $0.001 < P < 0.01$ and very highly significant if $P < 0.001$.

The correlation between the biochemical analyses and clinical results were determined by using the correlation coefficient of Pearson. The significance of the correlation was tested.

V Results

URINARY EXCRETION OF MONOAMINES

D o p a m i n e

Dopamine was excreted into the urine of parkinsonian patients $0.34 \pm 0.03 \mu\text{g}/\text{mg}$ of creatinine (Table 4). In the excretion of dopamine, there was no difference between all parkinsonian patients and the controls, who also excreted $0.33 \pm 0.06 \mu\text{g}/\text{mg}$ of creatinine. But the urinary excretion of dopamine in non-operated postencephalitic patients $0.16 \pm 0.04 \mu\text{g}/\text{mg}$ of creatinine was significantly ($P < 0.05$) lower than that of control patients. Within the parkinsonian group, there was no correlation between sex or age of the patients and the urinary excretion of dopamine. Urinary excretion of dopamine did not significantly correlate with severity of clinical signs, degree of disability of the patients or duration of the disease. However, urinary excretion of dopamine was significantly

Table 4. Urinary excretion of dopamine and HVA ($\mu\text{g}/\text{mg}$ of creatinine) of patients with Parkinson's disease and controls on three (1, 2 and 3) successive days. On the second day (2) is given 1.5 mg/kg b.w. of L-DOPA by intravenous injection. Mean $\pm$ S.E.M.

Group	D A			H V A		
	1	2	3	1	2	3
<u>Parkinsonian patients</u>						
Non-operated						
Idiopathic	0.43 $\pm$ 0.07 (32)	16.0 $\pm$ 3.8 (13)	0.69 $\pm$ 0.19 (31)	3.9 $\pm$ 0.7 (35)	8.6 $\pm$ 0.77 (33)	3.5 $\pm$ 0.4 (34)
Postencephalitic	0.16 $\pm$ 0.04 (8)	7.2 $\pm$ 2.1 (5)	0.30 $\pm$ 0.06 (6)	3.2 $\pm$ 1.0 (11)	6.7 $\pm$ 1.5 (8)	1.6 $\pm$ 0.2 (7)
All	0.38 $\pm$ 0.09 (40)	13.4 $\pm$ 3.8 (18)	0.63 $\pm$ 0.16 (37)	3.7 $\pm$ 0.6 (46)	8.2 $\pm$ 0.7 (41)	3.1 $\pm$ 0.4 (41)
Operated						
Idiopathic	0.31 $\pm$ 0.03 (27)	12.4 $\pm$ 3.0 (23)	0.51 $\pm$ 0.09 (25)	3.0 $\pm$ 0.2 (27)	10.6 $\pm$ 0.9 (27)	3.2 $\pm$ 0.3 (25)
Postencephalitic	0.28 $\pm$ 0.04 (11)	9.6 $\pm$ 2.0 (11)	0.35 $\pm$ 0.04 (11)	3.0 $\pm$ 0.5 (11)	7.2 $\pm$ 1.2 (11)	2.8 $\pm$ 0.3 (11)
All	0.30 $\pm$ 0.04 (38)	11.5 $\pm$ 1.6 (34)	0.46 $\pm$ 0.07 (36)	3.0 $\pm$ 0.3 (38)	9.6 $\pm$ 0.7 (38)	3.0 $\pm$ 0.2 (36)
All idiopathic	0.38 $\pm$ 0.04 (59)	13.7 $\pm$ 2.3 (36)	0.61 $\pm$ 0.11 (56)	3.5 $\pm$ 0.4 (62)	9.5 $\pm$ 0.7 (60)	3.3 $\pm$ 0.3 (59)
All postencephalitic	0.23 $\pm$ 0.03 (19)	8.9 $\pm$ 1.5 (16)	0.33 $\pm$ 0.05 (17)	3.1 $\pm$ 0.6 (22)	7.0 $\pm$ 0.9 (19)	2.3 $\pm$ 0.2 (18)
All parkinsonian patients	0.34 $\pm$ 0.03 (78)	12.2 $\pm$ 1.7 (52)	0.54 $\pm$ 0.09 (73)	3.4 $\pm$ 0.2 (84)	8.9 $\pm$ 0.2 (79)	3.1 $\pm$ 0.2 (77)
<u>Control patients</u>	0.33 $\pm$ 0.06 (33)	13.2 $\pm$ 2.0 (31)	0.45 $\pm$ 0.13 (34)	3.2 $\pm$ 0.5 (30)	9.0 $\pm$ 0.7 (31)	3.2 $\pm$ 0.4 (33)

Number of patients in parenthesis.

($P < 0.01$) lower in postencephalitic patients than in idiopathic ones. But there was no significant difference between patients who had undergone operation and those who had not.

H o m o v a n i l l i c a c i d

The basal urinary excretion of HVA (Table 4) was 3.4 ± 0.2 $\mu\text{g}/\text{mg}$ of creatinine in the group of parkinsonian patients and 3.2 ± 0.5 $\mu\text{g}/\text{mg}$ of creatinine in the control group. There was no significant difference between these groups. In patients with Parkinson's disease the urinary excretion of HVA did not show any significant correlation with age or sex of the patients or with the above-mentioned clinical variables.

3 , 4 - D i m e t h o x y p h e n y l e t h y l a m i n e

DMPEA appeared as a "Pink spot" in the paper chromatography for 15 patients from 37 parkinsonian patients and for 7 from 16 control patients. However, in the next step by two dimensional thin layer chromatography the "Pink spot" obtained divided into several spots without giving any spot in the same front of the authentic DMPEA

Table 5. Urinary excretion of DMPAA ($\mu\text{g/g}$ of creatinine) and 5-HIAA ($\mu\text{g/mg}$ of creatinine) of patients with Parkinson's disease and controls on three (1, 2 and 3) successive days. On the second day (2) are given 1,5 mg/kg b.w. L-DOPA by intravenous injection. Mean $\pm$ S.E.M.

Group	D M P A A			5 - H I A A		
	1	2	3	1	2	3
<u>Parkinsonian patients</u>						
Non-operated						
Idiopathic	24.7 $\pm$ 3.4 (27)	28.3 $\pm$ 4.2 (24)	24.7 $\pm$ 6.0 (24)	4.6 $\pm$ 0.4 (23)	5.2 $\pm$ 0.5 (22)	4.8 $\pm$ 0.6 (22)
Postencephalitic	12.9 $\pm$ 2.8 (5)	20.8 $\pm$ 10.4 (4)	8.2 $\pm$ 2.8 (5)	3.6 $\pm$ 0.3 (10)	5.0 $\pm$ 0.4 (8)	4.5 $\pm$ 0.8 (8)
All	22.9 $\pm$ 3.1 (32)	27.3 $\pm$ 3.8 (28)	21.8 $\pm$ 5.1 (29)	4.3 $\pm$ 0.3 (33)	5.0 $\pm$ 0.4 (30)	4.8 $\pm$ 0.4 (30)
Operated						
Idiopathic	16.9 $\pm$ 2.1 (19)	28.0 $\pm$ 4.9 (18)	16.6 $\pm$ 3.5 (17)	4.8 $\pm$ 0.6 (24)	4.5 $\pm$ 0.9 (21)	4.2 $\pm$ 0.5 (20)
Postencephalitic	19.6 $\pm$ 3.2 (7)	32.6 $\pm$ 9.6 (8)	26.9 $\pm$ 6.7 (7)	4.5 $\pm$ 0.4 (12)	4.0 $\pm$ 0.6 (11)	4.7 $\pm$ 0.7 (11)
All	17.6 $\pm$ 1.7 (26)	29.4 $\pm$ 4.3 (26)	19.6 $\pm$ 3.2 (24)	4.7 $\pm$ 0.4 (36)	4.3 $\pm$ 0.6 (32)	4.4 $\pm$ 0.4 (31)
All idiopathic	21.5 $\pm$ 2.2 (46)	28.2 $\pm$ 3.1 (42)	21.3 $\pm$ 3.8 (41)	4.7 $\pm$ 0.4 (47)	4.9 $\pm$ 0.5 (43)	4.5 $\pm$ 0.4 (42)
All postencephalitic	16.8 $\pm$ 2.8 (12)	28.6 $\pm$ 7.2 (12)	19.1 $\pm$ 4.8 (12)	4.1 $\pm$ 0.3 (22)	4.1 $\pm$ 0.4 (19)	4.6 $\pm$ 0.5 (19)
All parkinsonian patients	20.5 $\pm$ 1.9 (58)	28.3 $\pm$ 2.9 (54)	20.8 $\pm$ 3.1 (53)	4.5 $\pm$ 0.3 (69)	4.6 $\pm$ 0.4 (62)	4.6 $\pm$ 0.3 (61)
<u>Control patients</u>	21.8 $\pm$ 4.7 (18)	18.8 $\pm$ 4.9 (17)	14.6 $\pm$ 2.8 (17)	4.0 $\pm$ 0.5 (29)	4.8 $\pm$ 0.6 (28)	4.1 $\pm$ 0.7 (30)

3, 4 - D i m e t h o x y p h e n y l a c e t i c a c i d

The mean urinary excretion of DMPAA (Table 5) in parkinsonian patients was $20.5 \pm 1.9 \mu\text{g}/\text{mg}$ of creatinine. In the control group it was $21.8 \pm 4.7 \mu\text{g}/\text{mg}$. The difference of these values was not statistically significant. Within the parkinsonian group the basal urinary excretion of DMPAA in non-operated postencephalitic patients was significantly ($P < 0.05$) lower than in idiopathic ones. Nor was there any significant correlation between the urinary excretion of DMPAA and the above-mentioned other clinical factors.

N o r a d r e n a l i n e , a d r e n a l i n e , n o r -
m e t a d r e n a l i n e , m e t a d r e n a l i n e a n d
3 - m e t h o x y - 4 - h y d r o x y m a n d e l i c a c i d

Noradrenaline (Table 6) was excreted $42.3 \pm 8.6 \mu\text{g}/\text{mg}$ of creatinine by parkinsonian patients and $45.6 \pm 7.2 \mu\text{g}/\text{mg}$ of creatinine by controls. There was no significant difference between these values. Similarly, there was no significant difference between control and parkinsonian patients in excretion of VMA (Table 7), NMA or MA (Table 6).

Within the parkinsonian patients, urinary excretion of these compounds was of the same size in the stereotactically operated and non-operated patients, nor was there any significant difference between idiopathic or postencephalitic patients with Parkinson's disease.

Table 6. Urinary excretion of NA, A, NMA and MA ($\mu\text{g/g}$ creatinine). of patients with Parkinson's disease and controls on three (1, 2 and 3) successive days. On the second day (2) is given 1.5 mg/kg b.w. L-DOPA by intravenous injection. Mean $\pm$ S.E.M.

Group	NA	A	NMA	MA
<u>Parkinsonian patients</u>				
Non-operated				
Idiopathic	33.0 $\pm$ 7.5 (21)	10.4 $\pm$ 1.9 (23)	66.7 $\pm$ 38.6 (4)	7.9 $\pm$ 2.7 (3)
Postencephalitic	42.9 $\pm$ 12.8 (10)	6.7 $\pm$ 2.0 (10)	45.6 $\pm$ 22.0 (3)	13.7 $\pm$ 3.0 (3)
All	36.2 $\pm$ 6.5 (31)	9.3 $\pm$ 1.5 (33)	57.7 $\pm$ 22.6 (7)	10.8 $\pm$ 2.2 (6)
Operated				
Idiopathic	47.0 $\pm$ 8.5 (23)	8.7 $\pm$ 1.8 (23)	58.9 $\pm$ 16.3 (12)	12.9 $\pm$ 2.4 (14)
Postencephalitic	51.3 $\pm$ 14.2 (9)	6.8 $\pm$ 2.0 (9)	34.2 $\pm$ 6.9 (7)	7.1 $\pm$ 2.2 (5)
All	48.2 $\pm$ 7.2 (32)	8.1 $\pm$ 1.3 (32)	49.8 $\pm$ 10.8 (19)	11.4 $\pm$ 1.9 (19)
All idiopathic	40.3 $\pm$ 5.8 (44)	9.5 $\pm$ 1.3 (46)	60.9 $\pm$ 14.9 (16)	12.0 $\pm$ 2.0 (17)
All postencephalitic	46.9 $\pm$ 9.3 (19)	6.8 $\pm$ 1.4 (19)	37.6 $\pm$ 7.6 (10)	9.5 $\pm$ 2.0 (8)
All parkinsonian patients	42.3 $\pm$ 4.9 (63)	8.7 $\pm$ 1.2 (65)	51.9 $\pm$ 9.8 (26)	11.2 $\pm$ 1.6 (25)
<u>Control patients</u>	45.6 $\pm$ 7.2 (25)	7.1 $\pm$ 1.0 (25)	48.7 $\pm$ 6.7 (18)	15.1 $\pm$ 3.8 (18)

Number of patients in parenthesis.

5 - H y d r o x y i n d o l e a c e t i c a c i d

As can be seen in Table 5, the mean urinary excretion of 5-HIAA was $4.5 \pm 0.3 \mu\text{g}/\text{mg}$ of creatinine in parkinsonian patients and $4.0 \pm 0.5 \mu\text{g}/\text{mg}$ of creatinine in the corresponding controls. The difference was not statistically significant.

U r i n a r y e x c r e t i o n o f m o n o a m i n e s a f t e r L - D O P A - l o a d i n g

A single injection of 1.5 mg/kg b.w. of L-DOPA produced a significant ($P < 0.01$) increase in urinary excretion of both dopamine and HVA (Table 4). But there was no significant difference in the increase of excretion between the controls and the parkinsonian patients. Dopamine increased in both groups about 40 times but HVA increased only 2.5 - 3 times.

Within the parkinsonian group, increase in urinary excretion of dopamine and HVA after L-DOPA injection was of the same size in operated and non-operated as well as in postencephalitic and idiopathic patients. But the increase in the excretion of HVA was significantly lower in operated postencephalitic patients than in idiopathic ones ($P < 0.05$). However, there was no significant correlation between severity of the clinical signs, degree of disability of the patients or duration of the disease.

A single L-DOPA injection did not produce any significant differences in the urinary excretion of DMPAA (Table 5), of VMA (Table 7) or of 5-HIAA (Table 5), except DMPAA in operated idiopathic parkinsonian patients increased significantly ($P < 0.05$).

Table 7. Urinary excretion of VMA ($\mu\text{g}/\text{mg}$ of creatinine) of patients with Parkinson's disease and controls on three (1, 2 and 3) successive days. On the second day (2) is given 1.5 mg/kg b.w. L-DOPA by intravenous injection.
Mean $\pm$ S.E.M.

Group	V M A		
	1	2	3
<u>Parkinsonian patients</u>			
Non-operated			
Idiopathic	4.8 $\pm$ 0.4 (29)	6.0 $\pm$ 0.4 (27)	5.8 $\pm$ 0.4 (28)
Postencephalitic	3.7 $\pm$ 0.5 (10)	4.4 $\pm$ 0.7 (8)	4.7 $\pm$ 0.8 (8)
All	4.5 $\pm$ 0.3 (39)	5.6 $\pm$ 0.4 (35)	5.5 $\pm$ 0.4 (36)
Operated			
Idiopathic	4.4 $\pm$ 0.3 (30)	5.8 $\pm$ 0.6 (27)	5.0 $\pm$ 0.5 (26)
Postencephalitic	4.9 $\pm$ 0.5 (11)	5.2 $\pm$ 0.6 (11)	5.8 $\pm$ 0.8 (11)
All	4.6 $\pm$ 0.3 (41)	5.6 $\pm$ 0.5 (38)	5.2 $\pm$ 0.4 (37)
All idiopathic	4.6 $\pm$ 0.3 (59)	5.9 $\pm$ 0.4 (54)	5.4 $\pm$ 0.3 (54)
All postencephalitic	4.3 $\pm$ 0.4 (21)	4.9 $\pm$ 0.4 (19)	5.3 $\pm$ 0.6 (19)
All parkinsonian patients	3.6 $\pm$ 0.2 (80)	5.6 $\pm$ 0.3 (73)	5.4 $\pm$ 0.3 (73)
<u>Control patients</u>	4.3 $\pm$ 0.4 (30)	4.3 $\pm$ 0.3 (29)	4.0 $\pm$ 0.2 (30)

Number of cases in parenthesis.

ACID MONOAMINE METABOLITES IN THE CEREBROSPINAL FLUID

Concentration of homovanillic acid in the cerebrospinal fluid

The concentration of HVA in the CSF of the patients studied is summarised in Table 8. It can be seen that in the patients with Parkinson's disease the concentration of HVA, 14.4 ± 0.8 ng/ml, was significantly ($P < 0.001$) lower than that of the control subjects, 34.6 ± 1.9 ng/ml.

Table 8. Concentrations (ng/ml) of HVA and HIAA in parkinsonian and control patients in the basal state and after administration of probenecid. Mean $\pm$ S.E.M.

Group	Basal level		Response to probenecid	
	HVA	5-HIAA	HVA	5-HIAA
<u>Parkinsonian patients</u>				
All cases	14.4 ± 0.8 (157)	26.3 ± 1.5 (80)	57.4 ± 7.5 (63)	4.9 ± 2.5 (53)
Female	15.0 ± 1.2 (89)	26.8 ± 1.9 (52)	64.7 ± 9.6 (43)	3.5 ± 3.2 (37)
Male	13.7 ± 1.1 (68)	25.3 ± 2.6 (28)	41.9 ± 11.5 (20)	8.1 ± 4.1 (16)
Idiopathic	14.6 ± 0.9 (130)	26.2 ± 1.6 (67)	61.0 ± 8.5 (54)	5.1 ± 2.5 (9)
Postencephalitic	13.8 ± 1.8 (27)	26.5 ± 4.3 (13)	36.1 ± 12.1 (9)	3.6 ± 8.8 (44)
Non-operated	15.6 ± 1.2 (90)	28.3 ± 2.0 (45)	42.7 ± 9.0 (36)	0.3 ± 3.5 (21)
Thalamotomized	12.9 ± 1.1 (67)	23.8 ± 2.3 (35)	77.1 ± 12.1 (27)	8.3 ± 3.4 (32)
<u>Control patients</u>	34.6 ± 1.9 (63)	36.7 ± 3.8 (22)	69.6 ± 15.6 (21)	22.1 ± 10.0 (15)

Number of cases in parenthesis.

In non-parkinsonian control patients, there were no significant relationships between the content of HVA in the CSF and the age or sex of the patients.

Within the parkinsonian group, there were no correlations between sex or age of the patients and the level of HVA in the CSF. The concentration of HVA in postencephalitic patients did not differ from that of idiopathic patients. Furthermore, there were no significant differences between those patients who had previously undergone thalamotomy, and those who had not.

By analysing a possible relationship between the concentration of HVA and parkinsonian symptomatology, the content of HVA showed a significant ($P < 0.05$) negative correlation to severity of akinesia but no correlation to degree of rigidity or tremor. However, amount of HVA did not significantly correlate with the degree of disability of the patients or the duration of the disease.

Concentration of 5-hydroxyindole-acetic acid in the cerebrospinal fluid

The concentrations of 5-HIAA in the CSF are presented in Table 8. The parkinsonian patients had a significantly ($P < 0.05$) lower mean level of 5-HIAA, 26.2 ± 1.5 ng/ml, than in control subjects, 36.7 ± 3.8 ng/ml.

Correlation analyses showed a significant ($P < 0.05$) positive correlation between the 5-HIAA level in the CSF and the age of the

parkinsonian patients. However, no similar relationship was found in the control subjects. Furthermore, 5-HIAA level did not significantly correlate with parkinsonian symptomatology, the degree of disability of the patients or the duration of the disease. Thalamotomy had no significant effect on the concentration of 5-HIAA. Male control subjects had a significantly ($P < 0.01$) higher concentration of 5-HIAA in the CSF than corresponding female subjects, but female and male parkinsonian patients had an equal concentration of 5-HIAA. There was no difference between the mean age of both sexes in control or parkinsonian patients.

Effect of probenecid treatment on the concentration of homovanillic acid and 5-hydroxyindoleacetic acid in the cerebrospinal fluid

It can be seen from Table 8 that treatment with probenecid produced a statistically significant increase in the concentration of HVA in the CSF of both control, 69.6 ± 15.6 ng/ml ($P < 0.001$), and parkinsonian, 57.4 ± 7.5 ng/ml ($P < 0.001$), patients. However, the mean increase of HVA in parkinsonian patients did not significantly differ from that of non-parkinsonian patients.

In non-parkinsonian control subjects, the increase of HVA after probenecid treatment showed a significant positive correlation ($P < 0.05$) with the basal level of HVA in the CSF, but no correlation with the age or sex of the patients.

The mean rise of HVA after administration of probenecid in female patients with Parkinson's disease did not differ significantly from that of male patients. However, there was no significant relation between the rise in HVA following probenecid administration and the age of the patients. On the other hand, the mean increase of HVA in thalamotomized patients, 77.1 ± 12.1 ng/ml, was significantly ($P < 0.05$) higher than that in non-operated patients, 42.7 ± 9.0 ng/ml. In parkinsonian patients, the rise of HVA showed a significant negative correlation ($P < 0.05$ in both cases) with severity of akinesia and rigidity, and a tendency towards a positive correlation with tremor.

Treatment with probenecid caused a significant increase in the concentration of 5-HIAA in the controls, 22.1 ± 10.0 ng/ml ($P < 0.05$), but not in the parkinsonian patients, 4.9 ± 2.5 ng/ml. However, the differences in the increase of 5-HIAA in the CSF between control and parkinsonian patients was not statistically significant.

In non-parkinsonian patients the mean rise in 5-HIAA after probenecid treatment had a significant positive correlation ($P < 0.01$) with the baseline level of HVA in the CSF but not with that of 5-HIAA or with the age or sex of the patients.

In patients with Parkinson's disease the 5-HIAA response to probenecid did not show any significant correlation with the age or sex of the patients or with the above-mentioned clinical variables.

E f f e c t o f a s i n g l e i n j e c t i o n
o f L - D O P A o n t h e c o n c e n t r a t i o n
o f h o m o v a n i l l i c a c i d i n t h e
c e r e b r o s p i n a l f l u i d

In 24 control subjects the amount of HVA in the CSF was 29.0 ± 2.4 ng/ml. In 59 patients with Parkinson's disease the corresponding HVA level was 15.1 ± 1.4 ng/ml (Table 9). The difference was statistically significant ($P < 0.001$). Eight hours after the intravenous injection of 1.5 mg/kg b.w. of L-DOPA, the HVA value for the control series increased to 43.2 ± 5.4 ng/ml and for parkinsonian patients to 24.7 ± 3.3 ng/ml. This increase was significant in both the control ($P < 0.05$) and the parkinsonian group ($P < 0.01$) but was significantly smaller in parkinsonian patients than in the controls. Within the parkinsonian group there were no significant differences between the stereotaxic operated and non-operated patients. However, the increase of HVA after L-DOPA injection was significantly ($P < 0.001$) lower in postencephalitic parkinsonian patients than in idiopathic ones.

Table 9. The concentration of HVA (ng/ml) in the CSF of patients with Parkinson's disease and controls before (Pre) and eight hours after (Post) the intravenous injection of 1.5 mg/kg b.w. of L-DOPA.
Mean $\pm$ S.E.M.

Group	H V A (ng/ml)	
	Pre	Post
<u>Parkinsonian patients</u>		
Non-operated	15.9 $\pm$ 1.7 (40)	28.1 $\pm$ 4.7 (24)
Operated	13.5 $\pm$ 1.9 (19)	19.4 $\pm$ 4.1 (15)
Idiopathic	16.2 $\pm$ 1.5 (45)	26.8 $\pm$ 2.7 (35)
Postencephalitic	11.6 $\pm$ 2.2 (14)	6.4 $\pm$ 2.1 (4)
Total	15.1 $\pm$ 1.4 (59)	24.7 $\pm$ 3.3 (39)
<u>Control patients</u>	29.0 $\pm$ 2.4 (24)	43.2 $\pm$ 5.4 (21)

Number of patients in parenthesis.

CLINICAL RESPONSE TO SINGLE INJECTION OF L-DOPA

R i g i d i t y

The effect on rigidity of a single injection of L-DOPA and a placebo were measured and compared with each other. Table 10 shows that the parkinsonian patients improved greatly both on L-DOPA and on the placebo. By using Chi-Squares as a statistical treatment, the calculated scores did not reveal a significant difference between the L-DOPA and placebo groups. Nor was there any significant difference between non-operated and operated patients in regard to response to L-DOPA or the placebo.

Table 10. Distribution of the parkinsonian patients according to the changes in the rigidity and tremor after L-DOPA and placebo treatment.

State of patients	Rigidity				Tremor			
	$\frac{1}{2}$ h		6 h		$\frac{1}{2}$ h		6 h	
	L-DOPA	pl	L-DOPA	pl	L-DOPA	pl	L-DOPA	pl
Improved	19	20	20	18	16	15	11	13
No changes	14	14	12	14	16	15	20	17
Deteriorated	3	2	4	4	4	6	5	6

Table 11. Changes in the mean amplitude (mm) of the tremor oscillations after L-DOPA and placebo treatment. Mean $\pm$ S.E.M. (a. P < 0.05 compared to the pre-treatment values.

Group	Right			Left		
	pre	$\frac{1}{2}$ h	6 h	pre	$\frac{1}{2}$ h	6 h
L-DOPA						
Non-operated	6.6 ± 2.3	3.8 ± 1.1	5.8 ± 2.5	5.4 ± 2.5	2.0 ± 0.2	2.3 ± 0.3
Operated	8.5 ± 4.0	7.1 ± 3.3	6.0 ± 2.7	8.1 ± 3.0	6.0 ± 2.0	6.6 ± 2.0
Total	7.8 ± 3.0	6.1 ± 2.3	5.9 ± 2.0	7.2 ± 2.3	4.8 ± 1.4	5.3 ± 1.4
Placebo						
Non-operated	19.9 ± 11.6	16.5 ± 10.5	8.0 ± 3.7	7.1 ± 3.8	4.6 ± 1.9	3.5 ± 1.1
Operated	10.6 ± 5.1	5.8 ± 3.0	7.7 ± 3.7	9.2 ± 3.6	5.3 ± 2.1	7.0 ± 2.1
Total	13.4 ± 4.9	9.4 ± 3.8^a	7.8 ± 2.8	8.2 ± 2.7	5.1 ± 1.5^a	5.9 ± 1.5

T r e m o r

Table 11 shows the distribution of the parkinsonian patients according to changes in tremor. There was no significant difference between the L-DOPA and placebo groups. The response to L-DOPA was the same in non-operated and operated patients.

The tremorgram (Table 11), the mean amplitude obtained by an electrocardiographic recorder showed some decrease both after L-DOPA and placebo treatment, but the difference from the pretreatment value was significant ($P < 0.05$) only in the placebo group, 30 minutes after injection. However, the L-DOPA and placebo groups did not differ from each other in the mean amplitude of tremor either 30 minutes or 6 hours after the injection. Non-operated patients behaved similarly to those who had had undergone stereotaxic thalamotomy.

A k i n e s i a

The mobility of the patients improved significantly ($P < 0.05$) 6 hours after the injection of L-DOPA, but the placebo did not have the same effect. On the other hand, neither L-DOPA nor the placebo had any effect 30 minutes after the medication. Nor were any differences revealed in non-operated and operated patients in this respect (Table 12). The mean improvement scores of the performance test employed are presented in Table 12. A significant improvement occurred in the performance tests both 30 minutes and 6 hours after the L-DOPA and placebo injections. But the difference between the L-DOPA and placebo groups was not statistically significant.

Table 12. Changes in the mobility (walking time in 30 m) and performance tests (per cent from the pretreatment value) after L-DOPA and placebo treatment. Mean $\pm$ S.E.M.
 (a. $P < 0.05$ compared to the pretreatment values)
 (b. $P < 0.01$ " " " ")
 (c. $P < 0.001$ " " " ")

Group	Mobility			Performance tests				
	pre	$\frac{1}{2}$ h	6 h	right		left		
				$\frac{1}{2}$ h	6 h	$\frac{1}{2}$ h	6 h	
L-DOPA								
Non-operated	29.8 <u>±</u> 4.2	29.4 <u>±</u> 4.3	27.4 <u>±</u> 6.4	4.3 <u>±</u> 3.0	9.0 <u>±</u> 5.5	9.5 <u>±</u> 5.0	9.9 <u>±</u> 3.9 ^a	
Operated	28.3 <u>±</u> 1.8	29.3 <u>±</u> 2.5	27.3 <u>±</u> 2.0	10.0 <u>±</u> 2.7 ^b	9.7 <u>±</u> 3.1 ^b	8.5 <u>±</u> 1.7 ^c	3.5 <u>±</u> 2.6	
Total	29.0 <u>±</u> 2.1	29.3 <u>±</u> 2.3	27.3 <u>±</u> 2.8 ^a	7.3 <u>±</u> 2.3 ^b	9.4 <u>±</u> 3.0 ^c	9.0 <u>±</u> 2.6 ^b	6.6 <u>±</u> 2.4 ^b	
Placebo								
Non-operated	31.5 <u>±</u> 5.5	30.5 <u>±</u> 5.0	30.0 <u>±</u> 4.9	7.3 <u>±</u> 3.8	3.5 <u>±</u> 4.4	3.4 <u>±</u> 2.0	5.6 <u>±</u> 3.0	
Operated	29.8 <u>±</u> 2.2	30.2 <u>±</u> 3.2	30.1 <u>±</u> 3.1	5.6 <u>±</u> 2.0 ^b	2.7 <u>±</u> 2.4	7.1 <u>±</u> 1.7 ^c	4.6 <u>±</u> 2.4	
Total	30.5 <u>±</u> 2.8	30.3 <u>±</u> 2.7	30.1 <u>±</u> 4.8	6.4 <u>±</u> 2.1 ^b	3.1 <u>±</u> 2.3	5.4 <u>±</u> 1.3 ^c	5.0 <u>±</u> 2.0 ^a	

Table 13. Changes in the initiation and total reaction time (per cent from the pretreatment value) after L-DOPA and placebo treatment. Mean of changes in noise and light stimuli. (a. $P < 0.05$; b. $P < 0.01$).

Group	Initiation				Total reaction			
	right		left		right		left	
	$\frac{1}{2}$ h	6 h	$\frac{1}{2}$ h	6 h	$\frac{1}{2}$ h	6 h	$\frac{1}{2}$ h	6 h
L-DOPA								
Non-operated	11.7 $\pm$ 8.3	14.2 $\pm$ 7.9	1.1 $\pm$ 4.2	6.7 $\pm$ 4.8	13.3 $\pm$ 2.8 ^b	12.9 $\pm$ 4.2 ^a	8.4 $\pm$ 5.5	5.5 $\pm$ 2.0
Operated	6.3 $\pm$ 4.7	2.3 $\pm$ 5.6	4.8 $\pm$ 3.9	-1.8 $\pm$ 5.2	3.6 $\pm$ 4.2	2.2 $\pm$ 2.8	2.6 $\pm$ 3.5	-2.3 $\pm$ 4.3
Total	7.9 $\pm$ 4.0	5.9 $\pm$ 4.7	3.7 $\pm$ 3.0	0.7 $\pm$ 3.2	6.5 $\pm$ 3.4	5.4 $\pm$ 2.6	4.4 $\pm$ 2.9	0.02 $\pm$ 3.0
Placebo								
Non-operated	12.4 $\pm$ 7.3	15.2 $\pm$ 7.9	0.3 $\pm$ 5.5	12.0 $\pm$ 5.4	10.2 $\pm$ 6.4	15.9 $\pm$ 11.3	3.3 $\pm$ 9.3	13.8 $\pm$ 5.8
Operated	4.2 $\pm$ 4.3	0.1 $\pm$ 5.9	9.8 $\pm$ 3.4 ^a	1.5 $\pm$ 4.0	6.3 $\pm$ 3.7	0.3 $\pm$ 2.4	5.4 $\pm$ 3.4	0.2 $\pm$ 4.6
Total	6.7 $\pm$ 3.7	4.6 $\pm$ 4.9	6.9 $\pm$ 3.0 ^a	4.7 $\pm$ 3.2	7.5 $\pm$ 3.4 ^a	5.0 $\pm$ 3.9	4.7 $\pm$ 3.5	4.3 $\pm$ 3.8

Table 14. Effect of L-DOPA and placebo treatment on the blood pressure (mmHg) and pulse frequency (fr/min) of parkinsonian patients. Mean $\pm$ S.E.M.

	Injection				
	pre	post			
		5 min	15 min	30 min	6 h
Blood pressure (mmHg)					
L-DOPA					
systolic	151 $\pm$ 3	142 $\pm$ 5	137 $\pm$ 4	135 $\pm$ 4	149 $\pm$ 4
diastolic	92 $\pm$ 2	89 $\pm$ 2	87 $\pm$ 3	87 $\pm$ 3	89 $\pm$ 3
Placebo					
systolic	152 $\pm$ 4	142 $\pm$ 4	139 $\pm$ 4	139 $\pm$ 4	150 $\pm$ 4
diastolic	92 $\pm$ 2	89 $\pm$ 3	85 $\pm$ 3	88 $\pm$ 3	91 $\pm$ 2
Pulse frequency (fr/min)					
L-DOPA	82 $\pm$ 2	79 $\pm$ 2	78 $\pm$ 2	77 $\pm$ 2	82 $\pm$ 2
Placebo	81 $\pm$ 2	79 $\pm$ 2	76 $\pm$ 2	77 $\pm$ 2	81 $\pm$ 2

Table 15. Number of patients suffering from various side effects during and after L-dopa treatment.

	Injection						
	during	post				total	
		5 min	15 min	30 min	6 h	No	%
Nausea	4	13	6	2	3	17	47
Vomiting	1	8	4	3	1	11	31
Vertigo	3	1	1	1	2	7	19
Headache	6	8	8	8	8	12	33
Sweating	8	12	9	6	3	16	44
Anxiety	0	5	3	1	2	8	22
Total number of treated patients						36	

Measurement of initiation and total reaction time

The values for initiation time varied fairly extensively (Table 13). The only significant improvement was obtained in the left hand 30 minutes after the injection, and only in the placebo group. The difference between the groups was not significant. Between non-operated and operated patients, no difference was noted with regard to L-DOPA and placebo.

The total reaction time was significantly improved by L-DOPA on the right side of the non-operated patients after both 30 minutes ($P < 0.05$) and 6 hours ($P < 0.05$), but when the L-DOPA and placebo groups were compared with each other the difference was non-significant. On the other hand, the placebo had an improving effect in the total material after 30 minutes ($P < 0.05$), though there was no significant difference between the operated and non-operated patients.

Side effects

The occurrence of side effects is shown in Table 14 and 15. The most frequent side effect was nausea, which occurred in about 50 % of the L-DOPA-treated patients after 5 to 10 minutes. This symptom was noted both in non-operated and operated patients. Sweating and headache were also present in several cases.

Hypotonia occurred in five patients who were treated with L-DOPA, but decreased blood pressure was present in both groups immediately after the injection. All patients received the injection lying down.

VI Discussion

URINARY EXCRETION OF DOPAMINE AND HOMOVANILLIC ACID

As has been mentioned above in the introduction, previous investigations of urinary excretion of dopamine and HVA have been conflicting. In addition to the small series and several other factors have had a bearing on this. First of all, it must be kept in mind that many nutritional factors and drugs may influence catecholamine metabolism and determination methods (Euler et al.1956, Andersson et al.1958, Westfal and Watts 1964, Carruthers et al.1970). In addition, Caesar (1970) has reported that in normal humans alkalosis depresses urinary dopamine output. On the other hand, there are many claims that the patient's motor activity increases catecholamine excretion (Kärki 1956, Gunnie and Lidvall 1966). In view of these factors, the present author has tried to eliminate all sources of error as carefully as possible. The patient series were numerous enough to cover the whole spectre of different types

of parkinsonian patients and thus gave a good opportunity to correlate biochemical findings to different clinical variables.

The results of the present work showed that the basal urinary excretion of dopamine and its main metabolite HVA were roughly unchanged in patients with Parkinson's disease. This well agrees with the observations of Westlake and Tew (1966), Smith and Kellow (1969) and Schneider and Williams (1970). On the contrary, Barbeau et al. (1961, 1962), Bischoff and Torres (1962), Weil-Malherbe and van Buten (1969) and Metzel et al. (1970) have noted decreased urinary excretion of dopamine, and increased excretion has been reported by O'Reilly et al. (1965) in patients with extrapyramidal disorders characterised by excessive involuntary movements, including some parkinsonian patients, and Westlake and Tew (1966) shortly after stereotaxic thalamotomy. However, it must be noted that in the postencephalitic patients of our series the urinary excretion of dopamine decreased more significantly than did that of the idiopathic patients and non-operated postencephalitic patients had a significantly lowered urinary excretion of dopamine compared with controls. In the series of Barbeau et al. (1961, 1962) also decrease in urinary dopamine was greater in postencephalitic patients. The present results speak for the hypothesis that the different etiologies of the disease may have different effect mechanisms, and thus its reflections in urinary dopamine excretion are varying. It must be remembered that the concentration of dopamine in basal ganglia also decreased most in these postencephalitic patients (Ehringer and Hornykiewicz 1960 Hornykiewicz 1963). It could be presumed that in the postencephalitic form of the disease a specific factor causes so many changes outside the brain that the changes are reflected in the urinary monoamines. That this can happen was shown recently by den Hartog Jager (1970). He found in

the adrenal medulla of parkinsonian patients inclusion bodies which contained sphingomyelins, free fatty acids and polysaccharides and which resembled the Levy bodies (1912) found in the substantia nigra.

It has been suggested (Barbeau 1969) that the total catecholamine metabolism of the whole body is dependent on the quality of Parkinson's disease. Thus, hyperkinetic patients have increased and patients with hypokinesia decreased metabolism. But the result of the present investigation did not confirm this finding since it shows that urinary dopamine, HVA and other monoamines did not correlate to the parkinsonian symptomatology of the patients. It seems possible that the elevated catecholamine concentrations in hyperkinetic patients are associated with increased motility and could cause an elevated excretion of monoamines as a secondary phenomenon. This effect was eliminated in the present work, because the patients were at rest as completely as possible during the days the samples were taken.

Some evidence based on autopsy material has been presented that HVA behaves differently from dopamine in the brains of parkinsonian patients. It was noted that HVA did not decrease as much as dopamine (Bernheimer and Hornykiewicz 1964, 1965). It has also been reported (Barbeau 1968) that especially in the initial phase of parkinsonism dopamine concentration is normal or slightly decreased, but with akinesia clear dopamine decrease occurs. On the other hand, decreased urinary excretion of HVA appears later than that of dopamine. The present results show that the basal excretion of HVA corresponded to that of the control patients and did not correlate to the degree of severity of the disease or other clinical variables. This well agrees with our earlier observation (Rinne et al. 1967a). Other investigations have also shown that

there were no significant differences in the output of HVA (Greer and Williams 1963, Westlake and Tew 1966, Calne et al. 1969b) or other acidid or alcoholic catecholamine metabolites (Calne et al. 1969a), even though opposite opinions have also been presented (Simek 1968).

The unchanged basal urinary excretion of dopamine and HVA are corroborated by the findings that there was no significant change in the metabolism of exogenously administered L-DOPA into dopamine and HVA in patients with Parkinson's disease compared with controls. Except that in operated postencephalitic patients L-DOPA injection caused a significantly lower increase in the urinary excretion of HVA than in idiopathic ones. Thus, these results do not accord with the observation (Barbeau et al. 1962) that the metabolism of L-DOPA into urinary dopamine and 3,4-dihydroxyphenylacetic acid is generally lowered in parkinsonian patients. These roughly normal outputs of dopamine and HVA seem surprising with regard to the numerous proofs of the significance of dopamine in Parkinson's disease. However, urinary excretion within the normal range is understandable even when no general change in the metabolism of dopamine is involved, since the dopamine concentration in the brain represents only a small part of the total dopamine content in the body. The present results suggest that the changes in parkinsonism most often occur in the areas of dopamine and dopaminergic neurons in the brain. The significance of local changes in the dopaminergic substantia nigra neurons is also shown by the fact that in the brain of patients with hemiparkinsonismus, there was a more pronounced decrease of dopamine in the striatum opposite the side of symptoms (Barolin et al. 1964).

URINARY EXCRETION OF 3,4-DIMETHOXYPHENYLETHYLAMINE AND
3,4-DIMETHOXYPHENYLACETIC ACID

As has been mentioned in the introduction, the possible role of DMPEA and DMPAA metabolites in the parkinsonian syndrome is disputed. The "Pink spot" has been shown to contain several different components and very little if any DMPEA (Faurbye and Pind 1964, Creveling and Daly 1967). In the first place, development of the "pink spot" is influenced by many different factors. Diet, medication and bacterial flora are connected with its incidence (Stabenan et al. 1970, Thomas 1965, Studniz 1965). On the other hand, in a more accurate analysis the spot has been resolved into different compounds (Predescu 1968), of which small amounts have been identified as DMPEA (Creveling and Daly 1967, Watt et al. 1969). (1968) obtained completely negative results. The main part of the "Pink spot" has been noted to contain p-tyramine, the amount of which is dependent on the quality of food. Thus, patients on a carbohydrate diet excrete less tyramine than those on a normal diet (Vogel 1967). Other components of the "Pink spot" are such compounds as tyrosine, monoethylcadaverine, propionyl cadaverine and 5-hydroxytryptophane. Their excretion is influenced by food, so that glucose diet and the restriction of daily fluid intake decreases the excretion of these compounds (Kuehl et al. 1968, Watt et al. 1969). Cadaverine and the possible DMPEA have been noted to correlate to the intestinal bacterial flora (Perry et al. 1967, Studniz 1965). Intestinal antibiotics change the relation between the components of the spot and also decrease its occurrence frequency (Perry et al. 1967). Psychopharmacologic agents also play a role in the development of the spot. Phenothiazines and protixen increase the occurrence frequency of the "Pink spot" (Closs et al. 1967).

Although hypokinesia and rigidity are produced in laboratory animals by great doses of DMPEA (Barbeau 1966), it has no influence as a cause of psychoses, and in small doses it produced neither hypokinesia nor rigidity (Schulgin et al. 1966).

The present results also support the view that the "pink spot" is a secondary phenomenon, and thin layer chromatography did not reveal DMPEA in a finding obtained by paper chromatography. It is true that the patients had no restriction of food with protein, which would have excluded the possibility of p-tyramine.

As it has been noted in the introduction, there is some experimental evidence that DMPEA metabolizes into DMPAA (Friedhoff and Hollister 1966, Wagner et al. 1966). HVA is also metabolized into DMPAA. In accordance with some previous findings (Kuehl et al. 1966, Friedhoff and Furia 1967), it was found also in the present work that DMPAA was excreted in the urine of normal human subjects. Furthermore this excretion was of the same size in parkinsonian patients. The slightly significant difference between non-operated postencephalitic and idiopathic patients needs more establishments.

URINARY EXCRETION OF NORADRENALINE, ADRENALINE AND THEIR O-METHYLATED DERIVATIVES

With the normal excretion of noradrenaline and adrenaline and their methylated products in parkinsonian patients and controls, it must be kept in mind that both the synthesis and metabolism of noradrenaline and adrenaline occurs in the main outside the nervous system, in the adrenal medulla and sympathetic nervous system (Klavans 1968). Thus, the urinary noradrenaline and adrenaline concentrations and their methylated forms and VMA represent peripheral metabolism. The present results for the urinary excretion of monoamines correspond to the view that the monoamine metabolism outside of the brain is unchanged in parkinsonian patients, when the sources of error have been eliminated by experimental conditions as controlled as possible. The results of the present work are confirmed by some previous findings which also showed normal urinary excretion of noradrenaline and adrenaline in parkinsonian patients (Barneau et al. 1961, 1962, Nashold and Kirschner 1963).

In laboratory animals, it has been shown that besides a clear elevation of dopamine and HVA caused by exogenous L-DOPA, a relatively slight increase in the noradrenaline concentration of the brain occurs, and also that this is brought up more clearly only by small doses (Pletscher et al. 1970). The corresponding phenomenon has been pointed out in man as well by analysing autopsy samples of the brain of L-DOPA-treated parkinsonian patients (Rinne et al. 1971).

URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID

As to 5-HIAA, the present investigation also did not reveal any significant changes in urinary excretion, though contrary results have been reported (Barbeau 1963). The most important indolamine for the central nervous system is 5-hydroxytryptamine (5-HT), which is synthetised from tryptophan with an intermediate phase of 5-hydroxytryptophan. Normally, only one per cent of the tryptophan administered changes into 5-HT, and the rest is used for protein and nicotinamide synthesis (Garattini and Valselli 1965). Much more 5-HT is synthetised and metabolised in the intestine than e.g., in the central nervous system. Therefore it is understandable that urinary 5-HIAA reflects more the metabolism outside the brain than in the central nervous system (Jepson 1963). On the other hand, nutritional factors very easily effect on the excretion of this compound (Udenfriend et al. 1958). Thus, the normal urinary excretion concentrations of the present investigation support the view that the peripheral 5-HT metabolism is unchanged in parkinsonian patients.

ACID MONOAMINE METABOLITES IN THE CEREBROSPINAL FLUID

The origin of acid monoamine metabolites in the CSF is not exactly known, but an increasing amount of evidence indicates that they largely derive from the metabolism of monoamines in the brain and that their contents in the CSF closely reflect changes in the concentration of corresponding amines in the brain (Bartholini et al. 1966, Pletscher et al. 1967, Guldborg and Yates 1968, 1969, Andersson and Roos 1968, Eccleston et al. 1968, Moir et al. 1970). However, there may be many factors which could interfere with this relationship and which must be taken into consideration in interpreting the significance of concentrations of the acid monoamine metabolites in the CSF (Moir et al. 1970).

The results of the present study with a large number of parkinsonian patients clearly showed that the deficiency of dopamine in the substantia nigra striatum system is associated with decreased concentration of HVA in the CSF. This accords with lowered concentration of HVA in the lumbar (Johansson and Roos 1967, Bernheimer et al. 1966, Barolin and Hornykiewicz 1967, Rinne and Sonninen 1968a,b) and ventricular (Guldborg et al. 1967, Papeschi et al. 1970) CSF reported in other works. Furthermore, in the present investigation it was possible to show that the concentration of HVA in the CSF correlated with the severity of akinesia. Correspondingly, it has been found that the level of HVA in the ventricular CSF was significantly lower in parkinsonian patients with marked akinesia than in the other (Papeschi et al. 1970). It has been shown that the decreased concentration of dopamine and HVA in the striatum of parkinsonian patients is in good correlation with the degree of neuronal degeneration in the pars compacta of the substantia nigra (Hornykiewicz 1966). It is thus possible

that low levels of HVA in the CSF of patients with severe akinesia are due to fewer functioning substantia nigra neurones of these patients. The results of the probenecid test obtained in the present work give further support to this opinion. Accordingly, the therapeutic results obtained during long-term treatment with L-DOPA indicated that patients with more severe disease did not respond as well to L-DOPA (Rinne et al. 1970a,b).

The results of the present investigation proved in keeping with other findings (Johansson and Roos 1967, Olsson and Roos 1968, Gottfries et al. 1969) that the concentration of 5-HIAA in the CSF was also decreased in patients with Parkinson's disease, although this was less marked than that of HVA. However, low levels of acid monoamine metabolites in the CSF are not specific for Parkinson's disease, as reduced concentrations have been demonstrated in other neurologic (Barolin and Hornykiewicz 1967, Brody et al. 1970, Papeschi et al. 1970) and psychiatric (Bowers et al. 1969, Gottfries et al. 1969, Dencker et al. 1966) disease conditions.

There is evidence of some age-dependent variation in concentrations of acid monoamine metabolites in the CSF. In psychiatric and neurological patients Bowers and Gerbode (1968) have showed that there was a U-shaped relationship between age and content of 5-HIAA in the CSF, the youngest and oldest having higher levels than the middle-aged patients. Correspondingly, higher HVA levels were also found in the old age group. Gottfries et al (1970) did not found a similar relationship in healthy volunteers or psychiatric patients, but they did find a significant positive correlation between age and both 5-HIAA and HVA. In the present investigation, a corresponding correlation was found in parkinsonian patients only for 5-HIAA but not reveal any significant difference between the ventricular HVA and the age of parkinsonian patients.

However, it must be taken into consideration that in our patient material the age distribution was perhaps not great enough, since there was a lack of very young and old cases.

The reason for age related variations in the levels of acid monoamine metabolites in the CSF is not fully understood. It may be due to changes in the metabolism of brain monoamines or to alterations in elimination of acid metabolites from the CSF.

In agreement with other investigations (Bowers and Cerbode 1970, Gottfries et al. 1970), no relationship was found between sex and concentrations of HVA or 5-HIAA in the CSF, except for 5-HIAA in the control subjects. This difference in 5-HIAA of controls may be due to differences in age distribution, because there was an almost significantly higher mean age in male control subjects.

Stereotaxic surgical intervention has certain effects on the parkinsonian symptomatology of the patients. However, the results of the present work showed that previous stereotaxic thalamotomy did not affect the CSF. This is in accordance with the findings of unaffected level of HVA in the ventricular CSF (Papeschi et al, 1970) and urinary excretion of HVA (Rinne et al. 1967) and dopamine (Scheiden and Williams 1970). However, there was a significant difference in the HVA reponse to probenecid administration between operated and non-operated patients. The significance of this difference and possible factors leading to it will be studied.

Transport of acid monoamine metabolites from the brain to blood (Neff et al. 1964), and from the CSF to blood (Guldborg et al. 1966) will be inhibited by probenecid and thus accumulation of the metabolites in the CSF reflects the turnover of the parent amines in the brain (Moir et al. 1970, Tamarkin et al. 1970, Roos and Sjöström (1969). In agreement with dopamine deficiency of the parkinsonian brain it was found by Olsson and Roos (1968) that acid metabolites

in the CSF of patients with Parkinson's disease became elevated to a lesser extent than in controls. However, in our patient material this difference was not significant. This may be partly due to a great variance in the response to probenecid obviously due to too low level of probenecid in the blood. Tamarkin et al (1970) has recently shown the importance of high doses of probenecid for getting optimal results. In the other hand, the results of the present investigation showed that the elevation of HVA after probenecid administration correlated better with clinical variables than the simple basal level of HVA in the CSF. Obviously this elevation of HVA after probenecid administration is more sensitive test of functional dopamine deficiency in the parkinsonian brain than the basal level of HVA in the lumbar CSF.

The results of the present investigation showed that exogenously administered L-DOPA increased significantly the concentration of HVA in the CSF. But HVA concentration rose after L-DOPA injection significantly less in parkinsonian patients than in controls. Considering what was said earlier about the significance of HVA of CSF, this is evidence for the claim that a parkinsonian patient has less functional dopaminergic neurons than controls. However, it must be noted that after exogenous L-DOPA treatment HVA in the CSF may partly derive from the brain capillaries (Bartholini et al. 1971). Greatly increased HVA levels have been also found in the CSF of parkinsonian patients during long term treatment of high oral doses of L-DOPA (Curzon et al. 1970, Rinne et al. 1970a,b, Weinert et al. 1969). Thus these results obtained in L-DOPA loading test confirm the view that the functional dopaminergic neurons had decreased in the brain. Moreover the results of the CSF studies of the present study clearly indicated that the concentration of HVA in the CSF reflects changes in brain dopamine more accurately than does the urinary excretion of dopamine or HVA.

CLINICAL RESPONSE TO A SINGLE INJECTION OF L-DOPA

It is difficult to objectively quantitate the clinical symptoms in patients with Parkinson's disease. Firstly, it is not easy to find a good method, by which different symptoms may be measured by the same comparable scale. Secondly, many factors influence occurrence of the symptom, such as excitement due to the experiment, the time needed for the experiment and the peacefulness of the environment. In order to eliminate these defects, the author has tried to find tests which quantitatively measure single symptoms typical of parkinsonism. Thus, he decided to employ the tremor recording transducer (Clarke et al. 1966, Vas 1966) for measuring the severity of tremor accurately. In the same way, initiation and reaction time were measured by an apparatus (Barbeau 1966), in which the patient reacted to a light or sound stimulus, and the times corresponding to the initiation or performance were registered by an electrocardiographic apparatus.

To-day, there is strong evidence that the symptoms of parkinsonism are associated with a deficiency of dopamine in the extrapyramidal system (Ehringer and Hornykiewicz 1960, Hornykiewicz 1966). For the reason, the thought suggests itself that by using an agent which could be a substitute for the deficient or decreased neurotransmitter function, the corresponding symptom may also be eliminated. It has been noted that by administering, L-DOPA, the precursor of dopamine, the dopamine concentration in the brain is elevated. This was observed in animals (Pletscher et al. 1970, Bartholini et al. 1968, Butcher and Engel 1969, Everett and Bocherling 1970, Wurtman et al. 1970) and now also in man (Rinne et al. 1971). Correspondingly the urinary and CSF studies of the

present work showed increased metabolism of dopamine in the body after a single injection of L-DOPA. But a clear clinical effect on akinesia was found. The result can be explained by the overly short-term and weak influence of a single L-DOPA injection used. In this respect the results agree with some earlier observations (Greer and Williams 1963, McGreer and Zeldowicz 1964, Fehling 1966, Aebert 1967).

Although we have a great amount of evidence on the therapeutic effect of oral doses of L-DOPA in long-term treatment of parkinsonian patients (Conzias et al. 1969, 1969, Yahr et al. 1969, Barbeau 1969, Birkmayer 1969, Calne et al. 1969 a, b, Cotzias 1968, Godwin-Austen et al. 1969, Klawans and Garvin 1969, Siegfried et al. 1969, Tissot et al. 1969, Kaesar et al. 1969, Kaufmann et al. 1970, Mawdsley 1970, McDowell et al. 1970, Rinne et al. 1970a,b) there is only little knowledge about the exact mechanism of action of L-DOPA in parkinsonism. It is generally assumed that L-DOPA corrects the deficiency, and this is supported by the findings that L-DOPA itself seems to be pharmacologically inert (Carlsson et al. 1957 a, Blaschko and Cruschies 1960, Carlsson 1965) and that exogenously administered L-DOPA is mainly converted to dopamine and its degradation products, with only a minor proportion will undergoing β -hydroxylation to noradrenaline and further on to its metabolites (Bartholini and Pletscher 1968 b, Butcher and Engel 1969, Calne et al. 1969a, Pletscher et al. 1970, Sandler 1970). On the other hand, it has recently been emphasized that part of the clinical effect of L-DOPA may derive from the production and accumulation of some minor metabolites of L-DOPA, such as m-hydroxylated amines, 6-hydroxydopamine (Calne et al. 1969 b, Calne and Sandler 1970, Sandler 1970) and 3-O-methyldopa (Pletscher et al 1970).

VII Summary

In the present work the significance of monoamine metabolism in Parkinson's disease has been investigated. Monoamines and their metabolites were analysed in the urine and the cerebrospinal fluid (CSF). The effect of L-DOPA injection and probenecid treatment was studied in the same connection, as was the clinical response to a single injection of L-DOPA.

U r i n a r y e x c r e t i o n o f m o n o a m i n e s

The material consisted of 102 patients with Parkinson's disease and 48 non-parkinsonian control subjects. The parkinsonian patients comprised : 77 idiopathic, 25 postencephalitic patients and 50 of whom had undergone stereotaxic thalamotomy 2 years earlier on average.

The mean basal excretion of monoamines was calculated per creatinine. The values of excretion in the parkinsonian patients were $0.34 \pm 0.03 \mu\text{g}/\text{mg}$ for dopamine (DA), $3.4 \pm 0.2 \mu\text{g}/\text{mg}$ for homovanillic acid (HVA), $20.5 \pm 1.9 \mu\text{g}/\text{mg}$ for 3,4-dimethoxyphenylacetic acid (DMPAA), $42.3 \pm 4.9 \mu\text{g}/\text{mg}$ for noradrenaline (NA), $8.7 \pm 1.2 \mu\text{g}/\text{mg}$ for adrenaline (A), $51.9 \pm 9.8 \mu\text{g}/\text{mg}$ for normetadrenalin (NMA), $11.2 \pm 1.6 \mu\text{g}/\text{mg}$ for metadrenalin (MA), $3.6 \pm 0.2 \mu\text{g}/\text{mg}$ for vanilmandelic acid (VMA), $4.5 \pm 0.2 \mu\text{g}/\text{mg}$ for 5-hydroxyindolicacetic acid (5-HIAA). The corresponding control values were 0.33 ± 0.06 for DA, 3.2 ± 0.5 for HVA, 21.8 ± 4.7 for DMPAA, 45.6 ± 7.2 for NA, 7.1 ± 1.0 for A, 48.7 ± 6.7 for NMA, 15.1 ± 3.8 for MA, 4.3 ± 0.4 for VMA and 4.0 ± 0.5 for 5-HIAA. There were no significant differences between all parkinsonian patients and controls. However, there was a significant decrease of dopamine in the urine of non-operated postencephalitic patients ($P < 0.05$) compared to controls and in the urine of all postencephalitic patients compared to idiopathic ones ($P < 0.01$). In addition the basal urinary excretion of DMPAA in non-operated postencephalitic patients was significantly ($P < 0.05$) lower than in idiopathic ones. There was no significant correlation between the urinary excretion of these compounds and the clinical variables.

"Pink spot" (3,4-dimethoxyphenylethylamine, DMPEA) was detected by paper chromatography in urine from fifteen out of thirty-seven patients with Parkinson's disease and from seven out of sixteen control subjects. However, none of these urine samples showed a detectable amount of DMPEA when the "Pink spot" areas of the paper chromatogram were examined by thin layer chromatography.

A single injection of L-DOPA administration produced a significant ($P < 0.001$) increase in the urinary excretion of

dopamine and HVA and in addition ($P < 0.05$) in the urinary excretion of DMPAA in operated idiopathic patients but not in others. The increased values were generally of the same size in the controls and the parkinsonian patients. There was no significant difference between idiopathic and postencephalitic or between operated and non-operated patients with Parkinson's disease, except that increase in excretion of HVA was significantly lower in operated postencephalitic patients than in idiopathic ones.

Acid monoamine metabolites in the cerebrospinal fluid

The concentration of HVA and 5-HIAA was studied in the cerebrospinal fluid (CSF) of 157 patients with Parkinson's disease and 63 control subjects without extrapyramidal disorders. The effect of probenecid administration was studied in the same connection.

The mean concentration of HVA in parkinsonian patients 14.4 ± 0.8 ng/ml was significantly lower than that of control subjects 34.6 ± 1.9 ng/ml. In the parkinsonian patients, the content of HVA showed a significant negative correlation with the severity of akinesia. There was no significant effect of thalamotomy, sex or age of patients. There was no difference between idiopathic and postencephalitic patients.

The parkinsonian patients had significantly lower mean concentration of 5-HIAA, 26.2 ± 1.5 ng/ml than in controls, $36.7 \pm$ ng/ml. There was a significant positive correlation between the

5-HIAA level and the age of the parkinsonian patients, but no relation to clinical variables.

Treatment with probenecid caused a significant increase in the content of HVA and 5-HIAA in the CSF of both control and parkinsonian patients except for that of 5-HIAA in parkinsonian. However, the increase of HVA or 5-HIAA in patients with Parkinson's disease did not significantly differ from that in controls. Within the parkinsonian patients the rise of HVA showed a significant negative correlation with the degree of severity of the disease and disability of the patients as well as with the severity of akinesia and rigidity and a tendency to a positive correlation with tremor.

A single injection of L-DOPA produced in control subjects a statistically significant ($P < 0.01$) increase in the concentration of HVA in the CSF, and did the same in the patients with Parkinson's disease, but this increase was significantly smaller in the parkinsonian patients and in addition the increase of HVA in the CSF was significantly ($P < 0.001$) lower in postencephalitic patients than in idiopathic ones.

The clinical response to a single injection of L-DOPA

A controlled double blind trial of a single injection was carried out with 36 parkinsonian patients. The clinical effect was recorded half an hour and 6 h after the intravenous injection

of 1.5 mg/kg b.w. of L-DOPA or a corresponding amount of physiologic saline.

The amount of rigidity and tremor was scored on a five point scale and the tremor also with a tremor-recording transducer. Akinesia was assessed with various tests and by measuring mobility and initiation and total reaction time.

The best response to the single injection of L-DOPA was obtained with motility, the patients becoming more agile 6 h after L-DOPA treatment compared with the stage before L-DOPA injection and with the treatment of placebo. L-DOPA injection had no better effect on rigidity or tremor than the placebo. The total reaction time of the non-operated patients improved significantly after L-DOPA treatment but that of the operated patients did not.

The most usual side effect of L-DOPA injection was nausea, which occurred in 50 per cent of patients some of which experienced very severe hypotonia, too.

C o n c l u s i o n s

Results of the present study showed that monoamines and their metabolites were excreted in a normal amount in the basal state into the urine of almost all parkinsonian patients, except in the non-operated postencephalitic parkinsonian patients whose urinary excretion of dopamine was decreased compared with controls and in the postencephalitic patients compared with idiopathic ones. In the same way the effect of exogenously administered L-DOPA on the

urinary metabolites of monoamines was similar in the parkinsonian and non-parkinsonian patients. Except that in operated postencephalitic patients L-DOPA injection caused a significantly lower increase in the urinary excretion of HVA than in idiopathic ones. Thus monoamine metabolism outside of the brain in parkinsonian patients seems to be roughly normal, but it seems to be some differences between the two forms of the disease.

On the other hand, the amount of HVA in the CSF, both in the basal state and after L-DOPA or probenecid treatment, clearly showed decreased values compared with those of non-parkinsonian control subjects. Moreover these values showed a correlation to clinical variables. Thus concentration of HVA in the CSF better reflects the metabolism of dopamine in the brain than does urinary excretion, and suggests the decrease of functional dopaminergic neurons in the brain of the parkinsonian patients.

The clinical response to a single injection of L-DOPA suggested an anti-akinetic effect.

VIII Acknowledgements

The present study was in the main carried out at the Department of Neurology, University of Turku, and biochemical analyses at the Department of Anatomy, University of Turku.

The subject of my investigation was suggested to me by Professor U.K.Rinne, M.D., Head of the Department of Neurology, who throughout my work has given me inestimable support. I express my best thanks to him for his unceasing interest in my work and untiring helpfulness to me and particularly for his positive criticism during the preparation of manuscript, which was invaluable to in completing this study.

I also wish to express my best thanks to the Head of the Department of Anatomy, Professor M.Niemi, M.D., for making the facilities of his institute available to me.

I also thank Docent C.G.Nordström, Ph.D., and the staff of the Department of Endocrinal Laboratory, University Hospital of Turku, for their advice and their help in using their gas chromatographic

instrument.

My best thanks are due to my colleges at the Department of Neurology for their collaboration and friendship. In particular, I owe the nursing and technical staff a very great debt of gratitude for their continuous help in the collection of samples and in routine work throughout my study.

I wish to thank warmly the technical assistants, Mrs Raili Malinen and Miss Pirkko Lautsamo for their skillful assistance and for the great amount of work involved in performing the numerous analyses of this study.

I thank the staff of Medical Library of the University of Turku for their help in the collection of literature.

My sincerest thanks to Mrs Liisa Karkola, Fil.M., for her translations, Mr. Colin Black, B.A., for correcting the manuscript and to Mrs Kirsti Lundstedt for her typing of the manuscript and for her skillful assistance with the editorial work.

Financial support from Research Council for Medical Sciences (Finland), Duodecim Foundation, the Aaltonen Foundation and the Yrjö Jahnsson Foundation are gratefully acknowledged.

Turku, May 1971

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